Proceedings of the 2007 International Workshop on *Didymosphenia geminata*

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PROCEEDINGS OF THE 2007 INTERNATIONAL WORKSHOP ON *Didymosphenia geminata*

by

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ABSTRACT

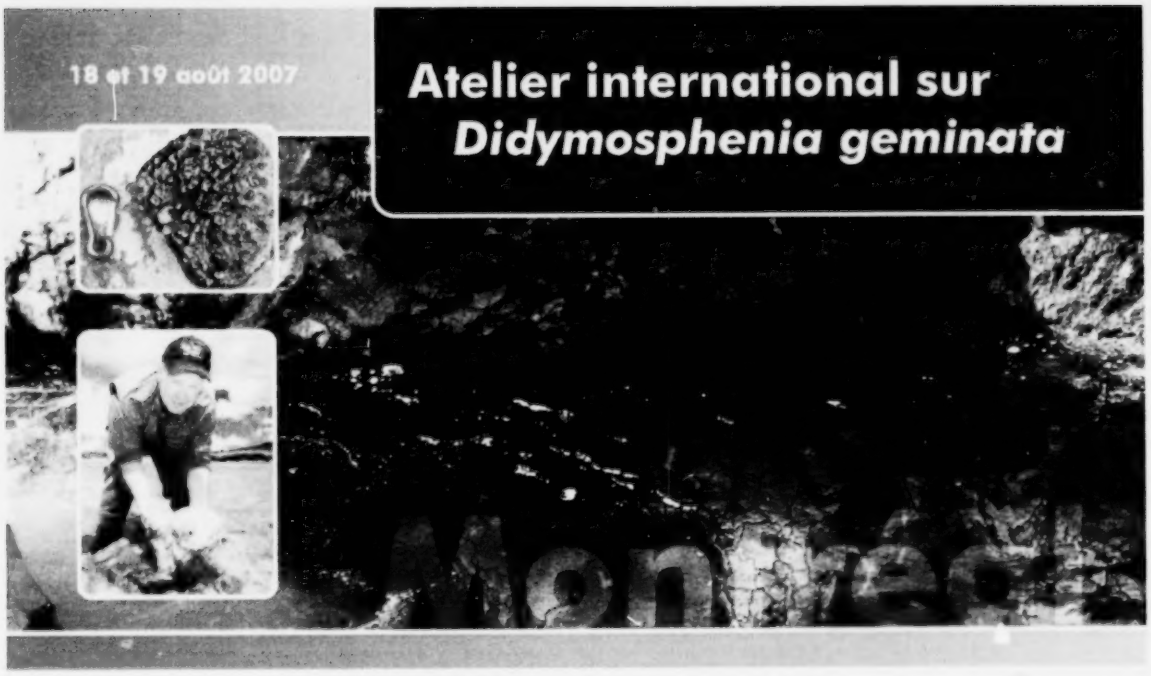
Bothwell, M. L. and Spaulding, S. A. (Co-Editors). 2008. Proceedings of the 2007 International Workshop on *Didymosphenia geminata*. Can. Tech. Rep. Fish. Aquat. Sci. 2795: xxxv + 58 p.

The 2007 - International Workshop on *Didymosphenia geminata*, sponsored by the Atlantic Salmon Federation, Environment Canada and Fisheries and Oceans Canada was held at the Montréal Convention Centre in Montréal, Quebec 18-19 August, 2007. Fifty-four registered delegates from Canada, the United States, Italy, the United Kingdom, Norway, New Zealand, Iceland, Peru and Austria representing research and management interests from federal, provincial and state governments, universities and non-governmental organizations were in attendance. On the first day a presentation was made on the current status of *D. geminata* blooms in Québec and the surveillance and public awareness programs run by the Québec Ministry of Sustainable Development, Environment and Parks. This was followed by presentations on the distribution, genomics, physiological ecology and environmental factors favoring *D. geminata* blooms in other parts of the world. Presentations on the second day of the Workshop focused on the impacts of *D. geminata* blooms on salmonid production in Norway, Iceland and in British Columbia. The workshop closed with an open discussion on the research needed to better understand the nature and impacts of didymo blooms worldwide and appropriate management actions needed to help control its spread.

RESUME

Bothwell, M. L. and Spaulding, S. A. (coéditeurs). 2008. Comptes rendus de l'Atelier international 2007 sur *Didymosphenia geminata*. Can. Tech. Rep. Fish. Aquat. Sci. 2795: xxxv + 58 p.

L'Atelier international 2007 sur l'algue *Didymosphenia geminata* s'est tenu les 18 et 19 août 2007, sous l'égide de la Fédération du saumon atlantique, d'Environnement Canada et de Pêches et Océans Canada, au Palais des Congrès de Montréal, Québec. Les cinquante-quatre participants inscrits à l'événement provenaient du Canada, des États-Unis, de l'Italie, du Royaume-Uni, de la Norvège, de la Nouvelle-Zélande, de l'Islande, du Pérou et de l'Autriche. Ils représentaient les milieux de la recherche et de la gestion des paliers de gouvernement fédéral, provincial et d'État de même que des universités et des agences non gouvernementales. La première journée de l'Atelier a débuté par une présentation sur la situation des proliférations de *D. geminata* au Québec et sur les programmes de dépistage de l'algue et de sensibilisation du public gérés par le ministère du Développement durable, de l'Environnement et des Parcs. Cette présentation a été suivie par d'autres exposés sur la répartition spatiale, la génomique, l'écologie physiologique et les facteurs environnementaux qui favorisent les proliférations de *D. geminata* ailleurs dans le monde. Les présentations de la seconde journée de l'Atelier ont ciblé les effets des proliférations de *D. geminata* sur la production des salmonidés en Norvège, en Islande et en Colombie-Britannique. L'Atelier s'est terminé par une discussion sur les besoins de recherche pour mieux comprendre la nature de Didymo et les effets de ses proliférations à travers le monde, et sur la gestion des interventions appropriées pour aider à contrôler sa dispersion.



18 et 19 août 2007

Palais des Congrès de Montréal

30e Congrès de la Société internationale de limnologie théorique et appliquée (SIL 2007)

Contenu de l'atelier de travail:

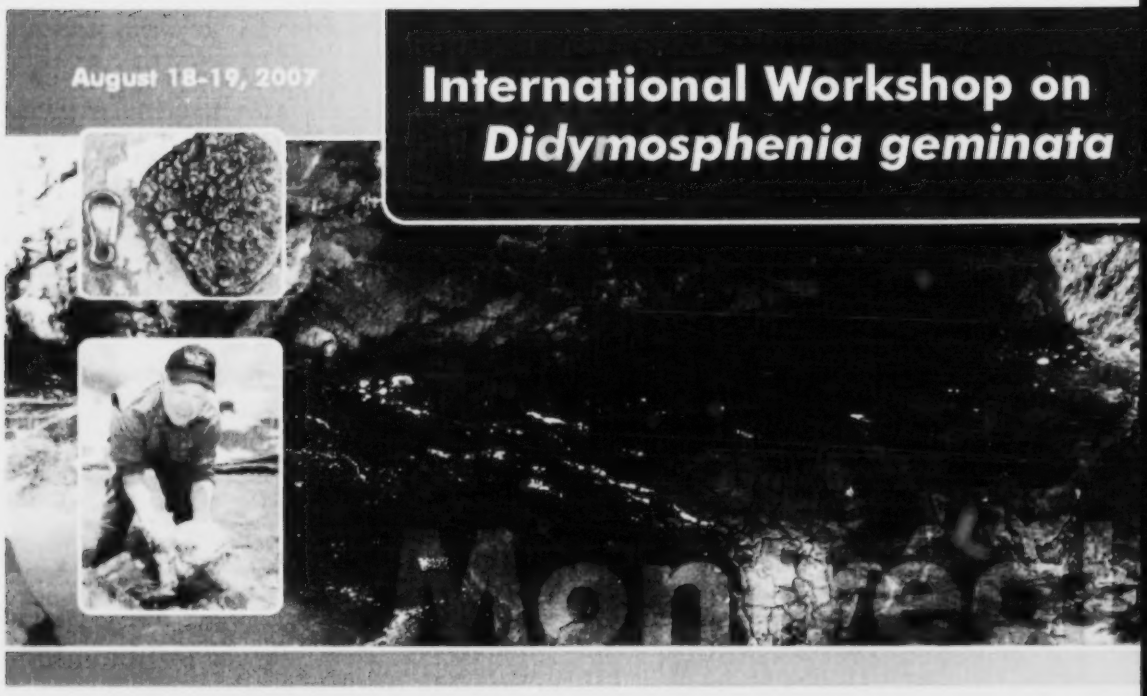
- Un groupe de scientifiques internationaux invités présenteront les résultats de leurs recherches récentes;
- Des spécialistes examineront les patrons des apparitions et des proliférations de *Didymosphenia* dans diverses régions géographiques afin d'y détecter des similarités et des différences;
- Des discussions sont planifiées pour améliorer la compréhension des facteurs qui contrôlent la répartition spatiale et l'abondance de *Didymosphenia*;
- Des progrès technologiques récents en génomique appliqués à la détection et à la dispersion de *Didymosphenia* seront présentés;
- Des résultats sur le rôle écologique de *Didymosphenia* dans les cours d'eau et ses liens avec la pêche sportive seront discutés;
- Nous espérons que les participants à l'atelier contribueront à la formulation de recommandations afin d'orienter les actions locales et internationales.

Pour information ou inscription (gratuite), contactez-nous au sil2007@JPdL.com

Environnement Canada

as-tsa

Québec



August 18-19, 2007

Palais des Congrès de Montréal - Montréal Convention Centre

30th Congress of the International Society of Theoretical and Applied Limnology (SIL 2007)

The workshop featured:

- An international group of invited scientists that will present the results of recent research
- Specialists will examine the patterns and history of *Didymosphenia* incursions and blooms from diverse geographic regions for their similarities and differences
- Discussions are planned to advance our of understanding factors controlling the distribution and abundance of *Didymosphenia*
- Recent advances in genomics techniques as applied to *Didymosphenia* detection and spread will be presented
- Results on the ecological role of *Didymosphenia* in streams and its relationship to fisheries will be discussed.
- We expect to resolve recommendations for local and international action based on the participation of attendees at the workshop.

For information or registration (free of charge), contact us at sil2007@JPdL.com

Environment Canada

Québec

FORWARD

Historical records indicate that *Didymosphenia geminata* (didymo) is likely native to lakes and rivers of Europe, Asia and North America and that blooms of didymo have occurred in river systems in Scandinavia, the British Isles, and Asia for many decades. It was not until the 1990's when unexplained blooms of didymo were first reported on Vancouver Island, British Columbia, Iceland and the Carpathian region of Poland that questions about the causes of these phenomena arose. Commencing in 2004, massive blooms of *D. geminata* in rivers on South Island New Zealand, where it had not been seen before, altered the direction of this discussion. The suggestion that a bloom-forming variant of didymo was inadvertently being transported between distant watersheds seemed possible. In 2006 *D. geminata* blooms were first reported in the Matapédia River, Québec and later confirmed in other vital Atlantic salmon rivers in the Lower St. Lawrence and Gaspé Peninsula regions. While a Special Session on didymo had been planned for the 2007 SIL Congress in Montréal prior to the reported outbreak in the Matapédia River, this Workshop was arranged in response to the new infestations and spread to adjacent states (Vermont, New Hampshire, and New York). The timing and venue for the International Workshop on *Didymosphenia geminata* could not have been better.

The Workshop participants brought a diverse international perspective and valuable expertise on didymo, and it is especially important that the results are published through a formal Proceedings. Despite the great interest in the distribution and impacts of didymo, little information on the distribution, trophic interactions, and magnitude of blooms is available in the scientific literature. Furthermore, the appearance of blooms in rivers of North America is now becoming an almost regular event. Unfortunately, each new appearance has been greeted with surprise from locals and fear from the media. We have an opportunity and the responsibility to make vital information available to scientists and managers so that appropriate responses to blooms are possible. At the same time, it is important to recognize that as managers request assistance with responding to the spread of didymo, progress to fill in gaps in knowledge seems painfully slow. There is a great deal that we do not know about didymo. One of the final activities of the International Workshop was to outline pressing management and research questions and we include these.

This Workshop and these Proceedings were made possible with financial support from the Organix Foundation to the Atlantic Salmon Federation (ASF), Environment Canada (EC) and Fisheries and Oceans Canada (DFO). Fred Whoriskey of the ASF administered the travel bursaries that allowed participants from around the world to come together in Montréal. André Talbot, John Carey and Fred Wrona of EC were champions who secured aide from the Canadian Government allowing us to host the Workshop at the Montréal Convention Centre. Ted Perry (DFO) facilitated publication of these Proceedings as a Canadian Technical Report of Fisheries and Aquatic Sciences and Marc Simoneau (Québec Ministry of Sustainable Development, Environment and Parks) graciously provided French translations for selected sections.

The International Workshop on *Didymosphenia geminata* demonstrated the extraordinary capacity of the concerned citizens of Québec, federal, Provincial and State government agencies, non-governmental organizations and university researchers to work productively together in common purpose to efficiently and timely communicate information on what didymo blooms are about, what the potential environmental concerns are, and what can be done to control the spread of didymo. All of the presenters and participants are responsible for the success of this endeavour.

Max Bothwell, Workshop Chair
Environment Canada
Pacific Biological Station
Nanaimo, British Columbia
CANADA

OF DIDYMO, ATLANTIC SALMON AND THE MANAGEMENT OF FEAR

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In August of 2006, news of the first documented bloom of didymo (*Didymosphenia geminata*) in a North American Atlantic salmon (*Salmo salar*) watercourse hit the Matapedia River, Quebec. This fell like a thunderbolt upon communities dependent on the salmon for their livelihoods. The Matapedia bloom eventually grew to extend along about 30 km of the river, covering up to 100% of the substrate with a carpet about 2.5 cm thick. Subsequent sampling in 2006 by Quebec government biologists detected drifting didymo cells in 6 of 12 neighboring Atlantic salmon rivers (Simard and Simoneau, this Proceedings). To the angling industry, it appeared that there was a real risk of the imminent development of didymo carpets very soon in many of the continent's premier Atlantic salmon rivers.

Atlantic salmon sports fisheries are big business in North America, generating about $175 million (Canadian) per year in economic activity and providing > 2000 jobs in rural areas where alternate employment opportunities are difficult to obtain (Whoriskey and Glebe 2002, MacIntosh 2001). Atlantic salmon populations in North America at present are depleted compared to historic levels, due to a combination of accumulated anthropogenic impacts (Watt 1988,1989), and more recently a decline in sea survival during the species' oceanic feeding migration (ICES 2007). Didymo blooms were widely viewed as a potential additional stressor for Atlantic salmon, and the Matapedia situation generated an intense burst of negative media reports. The Atlantic salmon world is well organized and wired, with multiple electronic channels of information exchange operating. News of the didymo bloom spread like wildfire.

Fisheries management has a little bit to do with managing fish, and a great deal to do with managing people, especially their fear for the preservation of their economic welfare and traditional ways of life. Fear is fed by an absence of reliable information. Local river managers and biologists had little information on hand about didymo at the time that the media storm hit, and the negative publicity surrounding the didymo bloom translated directly into cancellation of fishing bookings, fuelling the anxiety.

It was to address this climate of fear in a timely fashion that the Atlantic Salmon Federation (ASF) pledged its support to this workshop. The ASF had a number of hoped-for outcomes from the meeting, which perhaps not surprisingly dovetailed completely with the hopes of conference organizer Dr. Max Bothwell who has been thinking about didymo for many years. The first was to bring managers confronting didymo for the first time together with both didymo experts and managers with experience dealing with blooms in sport fishing rivers. Here we wished to rapidly transmit to the "naïve" managers the information they needed to understand the biology of didymo, its' present distribution, how it spreads, and what tools were available to help control the species. The second was to specifically review what was known about didymo impacts upon Atlantic salmon and other anadromous species, with an eye to assessing potential impacts upon the Atlantic salmon and its fishery. Finally, we hoped that the timely and accurate summary of information provided by the workshop would provide a common basis from which managers

newly confronting didymo blooms could build the customized communications plan that they needed. We felt that this could significantly reduce confusion in the media.

In our estimation, this workshop admirably met all of these goals. Notes from the meeting have circulated widely, and this proceedings document will provide an additional valuable record.

On behalf of the ASF, I thank the presenters and participants in the workshop for sharing their knowledge, and we offer our gratitude to the Organix Foundation, Environment Canada and the Government of the Province of Quebec for their sponsorship. Finally, Dr. Max Bothwell worked tirelessly on the workshop program, and has been an unflagging source of expert advice and collaboration in aid of those of us in North America who were encountering didymo for the first time. We deeply appreciate his efforts.

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SYNOPSIS
The 2007 International Workshop on *Didymosphenia geminata*

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BACKGROUND

The dramatic appearance of *Didymosphenia geminata* in 2004 in the Mararoa River of New Zealand's South Island sparked intense interest as to the cause and potential ecological impacts of blooms. While large masses of this organism had been observed in several regions around the world, the Mararoa River blooms marked the recognition of *D. geminata* as an invasive organism. Awareness of the invasive nature of *D. geminata* prompted a fresh look at blooms internationally and initiated an acute interest in the role of this diatom in influencing aquatic communities and ecosystems. Based on its invasive habit, *D. geminata* was labelled an undesirable, alien species by Biosecurity New Zealand. Funding was then secured in New Zealand for research on best approaches to detect, contain and control the spread of *D. geminata.* As a result, Biosecurity New Zealand initiated an aggressive program to gain an improved understanding of potential deleterious impacts on river food webs, sports fisheries and endemic species. Research in New Zealand provided much of the fundamental work on *D. geminata*, including the first sequencing of *D. geminata* 18S rRNA and discovering the diatom's ability to survive outside aquatic habitats, including surviving within the felt soles of waders.

Two years later, in 2006, the onset of *D. geminata* blooms in rivers in eastern Québec ignited regional concern about potential damage to Atlantic salmon stocks, as well as aesthetic and ecologic concerns. The International Workshop on *Didymosphenia geminata* provided a forum for a panel of scientific experts assembled from around the world to communicate to interested parties in Québec and adjacent Provinces and States the:

1. distribution, detection, genomics, physiological ecology and environmental factors favoring blooms;
2. potential impacts of blooms on salmonid fisheries;
3. approaches to management and control available to governmental agencies;
4. recommendations for a research agenda to improve understanding of blooms and focus agency response.

The timing of the Workshop to correspond with the 2007 International Meeting on Theoretical Limnology (SIL) was propitious; the Québec Ministry of Sustainable Development, Environment and Parks (MDDEP) had just confirmed the reoccurrence of didymo in the Matapédia River and appearance of didymo cells in six adjacent rivers on the Gaspé Peninsula (Isabelle Simard and Marc Simoneau, MDDEP). In addition, only weeks before the Workshop, *D. geminata* blooms in Vermont, New Hampshire and upper New York State were reported. At the time of the meeting in August 2007, managers in Quebec and several States in the US were in urgent need of current information and the meeting was well attended.

DISTRIBUTION, DETECTION, GENOMICS, PHYSIOLOGICAL ECOLOGY AND ENVIRONMENTAL FACTORS FAVORING BLOOMS

Didymosphenia geminata has a wide distribution best documented in Europe, Asia and North America leading to the assumption that it is probably native to those regions. Prior to 2004, reports of *D. geminata* in the Southern Hemisphere were rare and not always reliable (Kilroy 2007, Spaulding and Elwell 2007). While blooms of *D. geminata* in New Zealand have garnered wide attention in the past two years, presentations by Brian Whitton (UK) and Eli-Anne Lindstrøm (Norway) reminded Workshop attendees that *D. geminata* has been forming blooms in some rivers in Britain and in Scandinavia for decades, if not centuries, i.e. *D. geminata* blooms are not a new phenomenon in the Old World.

Didymosphenia geminata blooms in New Zealand are almost certainly the result of a species introduction (Kilroy et al. 2004). The rapid spread of *D. geminata* among catchments on the South Island and the magnitude of the biomass accumulations in some of those rivers typify invasion biology when a species is introduced to a suitable habitat for the first time. However, the causes of *D. geminata* blooms elsewhere in the world are more problematic since it is native to many of these areas. *D. geminata* appears to be expanding its range into new geographic regions and habitat types. *D. geminata* blooms are now occurring at lower latitudes and in warmer water environments than were previously thought to be unfavourable for this organism (Spaulding and Elwell 2007, Kumar et al. 2008). Whether this shift is associated with a new introduced strain of the species is unknown. However, in regions where *D. geminata* is native the pattern of spread of blooms between watersheds documented in the last decade (Vancouver Island (Bothwell et al. 2006) Iceland (Jonsson et al. 2000), South Dakota, Colorado (Spaulding and Elwell 2007) suggests invasive movement.

One of the top priorities of Biosecurity New Zealand for the *D. geminata* program was the development of a sensitive and accurate molecular tool for screening catchments for the presence/absence of *D. geminata.* Such a tool was developed by the Craig Cary lab at University of Waikato / University of Delaware. The Cary lab sequenced 18S rRNA from *D. geminata* and designed several didymo-specific PCR primers that can reliably distinguish the species from closely related species (Cary et al. 2007 and this Proceedings). A quantitative real-time polymerase chain reaction (QRT-PCR) protocol was developed that allows rapid quantification of *D. geminata* in river water samples (Cary et al. 2007). The method is sensitive, accurate and robust and is now in routine use for incursion assessments in New Zealand. The same assay has been successfully tested on a range of international samples. This approach to screening rivers

for *D. geminata* applied to broad geographic regions might clarify factors determining its distribution.

Cary et al. (2007 and this Proceedings) have also begun investigating the use of molecular markers in the internal transcribed spacer (ITS) regions of the 18S rRNA gene to distinguish between populations of *D. geminata* in different geographic regions. The preliminary findings indicate marked separation between European and North American strains of *D. geminata.* Furthermore, the work indicates a close affiliation of North American and New Zealand populations. Additional work continues using random amplified polymorphic DNA markers (RAPDs) and inter simple sequence repeat (ISSRs) to establish finer scale variation. Cary's lab, in conjunction with Biosecurity New Zealand, has issued a worldwide request for *D. geminata* samples to be submitted for genomic analysis for a phylogeographic study of *D. geminata.* Workshop attendees were urged to participate and handouts describing procedures for collection, handling and submitting samples were available.

Several presentations at the Workshop discussed environmental factors associated with abundant *D. geminata* growth in rivers and physiological attributes that might favor *D. geminata*'s success in competition with other algal species. Stability of flow and low turbidity were reaffirmed as important factors contributing to *D. geminata* abundance in Alberta rivers (Kirkwood et al. 2007 and this Proceedings). These two conditions typify lake and reservoir outlets and help explain why these environments often support high *D. geminata* biomass.

D. geminata has long been considered a cold water species, but more recent observations in warmer rivers (at least during summer) have challenged that assumption (Bothwell et al. 2006, Spaulding and Elwell 2007). An intriguing finding from Cathy Kilroy's presentation was that *D. geminata* is never in streams or rivers anywhere in the world where the minimum mean air temperature in the coldest month of the year is above 5°C. The implication that there is some physiological requirement for cold water for at least part of the year in order for *D. geminata* to thrive is completely new and clearly calls for more detailed examination of global records and experimental testing (Kilroy et al. 2007).

Water chemistry also exerts strong control on *D. geminata* presence and abundance. Surveys in Norway show that *D. geminata* requires minimum concentrations of Ca (>2 mg/L) and S (>2.5 mg/L). Gradients in Ca and S explain much of the broad scale distribution pattern of *D. geminata* in Norway (Lindstrøm and Skulberg 2007 and this Proceedings). Observations in Sierra Nevada streams corroborate this finding, with higher Ca and S concentrations being one of the factors distinguishing didymo streams from non-didymo streams (Rost et al. 2007).

D. geminata has historically been associated with pristine water conditions, i.e, very low nutrient levels, but observations in Norway and Alberta indicate that moderate levels of inorganic P enrichment in streams may stimulate *D. geminata* growth (Lindstrøm and Skulberg this Proceedings; Bowman this Proceedings). Alkaline phosphatase, a cell-surface enzyme that allows cells to utilize extracelluar dissolved organic phosphorus (DOP), has been shown to be localized is recently secreted stalks (Ellwood and Whitton 2007). This enzyme might provide didymo a competitive advantage in low nutrient environments where the majority of dissolved phosphorus is present as mono- and diphosphate esters. It was suggested that recent blooms of *D. geminata* in some regions might be associated with elevated DOP related to changing climate conditions (Whitton and Ellwood this Proceedings). Levels of DOP in rivers should be

considered when assessing environmental factors controlling didymo abundance. Not withstanding this observation, *D. geminata* remains a pollution sensitive species with abundance declining when total organic carbon >6.5 mg/Land TP >20 µg/L, at least in Norwegian waters (Lindstrøm and Skulberg this Proceedings).

POTENTIAL IMPACTS OF BLOOMS ON SALMONID FISHERIES

Critical information about the status and productivity of salmonid populations in rivers historically impacted by *D. geminata* was presented at the Workshop. Because of the recent infestation in the Matapédia River, Québec, potential impact on Atlantic salmon (*Salmo salar*) was a paramount concern to many attendees. Lindstrøm and Skulberg (2007 and this Proceedings) provided evidence for *D. geminata* blooms dating back 150 years in Norwegian rivers that have remained highly productive for Atlantic salmon. Some of the most productive Atlantic salmon rivers in Norway have also been the sites of the most extensive *D. geminata* blooms in the country (Lindstrøm and Skulberg this Proceedings).

D. geminata blooms have occurred in Scandinavian rivers for many years and it might be argued that impacts on Atlantic salmon would be different in regions recently impacted by such blooms. However, evidence from more recent *D. geminata* infestations in Iceland supports the Norwegian experience. *D. geminata* blooms in Iceland's rivers commencing in 1994 have had no obvious negative impact on *Salmo salar* populations (Jonsson et al. 2000; Jonsson et al. this Proceedings).

An analysis of extensive historical records for returns and productivity of three species of Pacific salmonids was presented. Coho salmon (*Oncorhynchus kisutch*), chum salmon (*O. keta*), and steelhead trout (*O. mykiss*) populations that rear in rivers on Vancouver Island that were affected by *D. geminata* blooms were compared to populations in rivers without *D. geminata* (Bothwell et al. this Proceedings). For all three species there was either no significant impact on escapement and productivity, or there was a positive effect that was not consistent across all rivers (Bothwell et al. this Proceedings).

Interactions between *D. geminata* blooms and fish are dependent on species life history, feeding ecology and interactions with other species. There are no reported negative affects of *D. geminata* on diadromous fishes, those fishes that spend only a portion of their life in freshwater. We might ask if the same is true of fish populations that spend their entire life history in inland waters. Evidence presented at the Workshop implies a strong negative impact of *D. geminata* on resident brown trout (*Salmo trutta*). In Rapid Creek (South Dakota), a precipitous decline in the abundance of adult brown trout coincided with the commencement of *D. geminata* blooms in 2002 (Shearer and Erickson 2006, Larson and Carreiro this Proceedings). While the collapse of the brown trout fishery in Rapid Creek, SD is compelling, evidence of *D. geminata* impacts on brown trout in other parts of the world is still lacking.

An analysis of invertebrate communities in Rapid Creek suggested that a feeding bottle-neck may have resulted in the abundance of young-of-the-year to age-1 fish coupled with the absence of larger adults. In that river, *D. geminata* abundance resulted in a lower percentage of Ephemeroptera, Plecoptera and Trichoperta (EPT) community that are more important for larger-sized trout (Larson and Carreiro this Proceedings). A similar result was found in a comparison of a didymo and a non-didymo stream in the Sierra Nevada (Rost et al. this Proceedings). That

study also found suggestive evidence through stable isotope analysis that trout in a *D. geminata* stream were less dependent upon algal carbon than trout in an adjacent non-didymo stream (Rost et al. this Proceedings). Benthic community composition studies in New Zealand found similar shifts in the percent EPT taxa, but on an absolute level the abundance of all insect taxa were still greater in *D. geminata* bloom areas compared to non-affected river reaches (Larned et al. 2007). Food webs, by their very nature, are complex and interactive. The effects of *D. geminata* on trophic dynamics of streams will likely vary with many factors, including the extent of coverage, mat thickness and bloom duration. Because of this complexity, discrepancies between different studies are not surprising.

APPROACHES TO MANAGEMENT AND CONTROL AVAILABLE TO GOVERNMENTAL AGENCIES

Prevention

Biosecurity New Zealand reviewed options for containment of *D. geminata* early in its discovery on the South Island. Increasing the level of public awareness was determined to be the most important action based on the following rationale: 1) Eradication or control of a microscopic alga is not likely in the short term, 2) Cells of *D. geminata* were likely to be present beyond the known impacted waterways, 3) Restricting user access to rivers would not address the risk of spread by birds and other animals, 4) Appropriate cleaning methods for river equipment have been developed, 5) Public awareness would be more likely to gain widespread compliance than regulations and closures.

Control

Investigation of ten potential control agents (algaecides/biocides) was initiated through experimental trials (Jellyman et al. 2006). The control agents were rated based on their effectiveness on causing cell mortality and degradation of biomass. In addition, ecosystem impacts and risks, feasibility of application, cost, and duration for effective control were considered. A second phase of testing was based on the results from the first study. Trials in artificial channels showed that a chelated copper compound (Gemex™) was the most effective in killing *D. geminata* cells and minimizing effects on non-target species (Clearwater et al. 2007a). Finally, a control and eradication experiment was carried out in an impacted stream (Clearwater et al. 2007b). The results indicated that treatment could be effective in reducing, but not eradicating, the population of *D. geminata*. A number of spring-fed creeks that are tributaries of *D. geminata* affected rivers were found to lack populations of *D. geminata,* despite their exposure to colonization (Sutherland et al. 2007). This study, to compare the survival of *D. geminata* on artificial substrates in rain-fed rivers vs. spring-fed creeks found poor survival of cells in spring-fed creeks. Although the factors, or combination of factors, influencing survival was not determined, this line of inquiry should be followed in future work.

Monitoring and surveillance

Genetic fingerprinting tools for detecting the presence of *D. geminata* in watersheds was developed and implemented in New Zealand (Cary et al. 2007). The technique includes field procedures for efficient collection of microscopic cells and identification of a species – specific

DNA sequence that allows the differentiation of *D. geminata* from other species of diatoms in New Zealand and international rivers. Furthermore, provisional genetic markers were determined that indicate that cells of *D. geminata* in New Zealand were likely introduced from North America.

Public education

In the past 2 years many government and non-government agencies have published information about *D. geminata* and recommendations for cleaning of aquatic sports and fishing gear to prevent the inadvertent spread of *D. geminata*.

BioSecurity New Zealand
http://www.biosecurity.govt.nz/didymo
Quebec MDDEP
http://www.mddep.gouv.qc.ca/eau/eco_aqua/didymo/didymo-en.pdf
New Brunswick
http://www.gnb.ca/0254/FAQDidymo-e.asp
US Environmental Protection Agency
http://www.epa.gov/region8/water/didymosphenia/
NEANS
http://www.invasivespeciesinfo.gov/index.shtml

RECOMMENDATIONS FOR A RESEARCH AGENDA AND AGENCY RESPONSE

Large growths of *Didymosphenia geminata* are of concern in stream ecosystems and there remain research needs to resolve basic and applied management questions.

1) There is a repeating pattern of the appearance of nuisance blooms in North America, beginning with Vancouver Island, British Columbia in 1989. With each successive report in Canada (Alberta, Yukon, Québec) and the United States (Colorado, Montana, Missouri, Arkansas, New Hampshire, Pennsylvania, Vermont, New York) managers are faced with the same problem of little information on the biological and environmental factors that trigger the formation of blooms. The temporal and spatial extent of mats could be mapped in association with use by human vectors. Such data could address the complex interaction of hydrologic, chemical, climatic and human roles in bloom development.

2) Preliminary work on population genetics suggests that New Zealand organisms arrived from western North America, but it is not known if a "nuisance form" of *D. geminata* represents a new, or unique strain. Molecular markers for populations of *D. geminata* need to be further developed to a) determine the relatedness of populations across the globe and b) establish if there is a unique genetic form responsible for invasive behavior. An effort to gather samples at recent international meetings (ASLO February 2007, SIL August 2007) was initiated by Biosecurity New Zealand and requires further support.

3) Resolution of the historical abundance of *D. geminata* is possible through existing archived samples and lake sediment records. Diatom samples that have been collected, processed, and archived may be available for *D. geminata* specific analysis. That is, slides can be examined for

D. geminata abundance. In most studies, the abundance of diatoms is based on total numbers of diatom cells and is heavily biased toward the small cells that dominate most counts. Such an approach is straightforward and low in cost to accomplish. A second method to address historical abundance is to identify rivers that experience nuisance blooms and empty into lakes or reservoirs, depositing the siliceous cells into lacustrine sediments. In these sites, the surface sediments and abundance of cells can be correlated to reconstruct the historical abundance of the river drainage.

4) The abundance of *D. geminata* is related to trophic level impacts, that is, with greater abundance there are greater changes in algal and invertebrate communities. Several studies have documented the changes in algal and invertebrate communities, yet the quantitative and long-term implications are not known. In particular, both algal and invertebrate communities are low in diversity with high *D. geminata* abundance compared to those without *D. geminata*.

5) Despite the importance and impact of this organism, many aspects of its basic biology and life history are not known. Although sexual reproduction has been documented in the literature, it has not been observed in recent blooms. Vegetative cell division within the diatoms results in successively smaller generations of cells and a crucial part of the diatom life cycle is the restoration of maximum cell size through formation of auxospores. Investigation of the cell size distribution within a population over time would provide fundamental knowledge about the species, even if sexual stages prove difficult to observe.

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SYNOPSIS

L'ATELIER DE TRAVAIL INTERNATIONAL 2007 SUR *DIDYMOSPHENIA GEMINATA*

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CONTEXTE

L'apparition spectaculaire de *Didymosphenia geminata* en 2004, dans la rivière Mararoa de l'Île du Sud de la Nouvelle-Zélande, a suscité un vif intérêt quant aux causes et aux impacts écologiques potentiels des proliférations de cette algue. Bien que de grandes masses de cet organisme aient été observées dans plusieurs régions du monde entier, ce sont en particulier les proliférations de la rivière Mararoa qui ont marqué la reconnaissance de *D. geminata* comme un organisme envahissant. La prise de conscience de la nature envahissante de *D. geminata* a incité un nouveau regard sur ses proliférations à l'échelle internationale et entraîné un intérêt marqué sur le rôle de cette diatomée et son influence sur les communautés et les écosystèmes aquatiques. En raison de son caractère envahissant, *D. geminata* a été étiquetée espèce exotique indésirable par Biosecurity New Zealand. Cette reconnaissance a assuré le financement pour la recherche en Nouvelle-Zélande afin de développer les meilleures approches pour détecter, contenir et contrôler la dispersion de *D. geminata.* En conséquence, Biosecurity New Zealand a introduit un programme agressif pour acquérir une meilleure compréhension des effets délétères potentiels sur la chaîne alimentaire des rivières, la pêche sportive et les espèces indigènes. La recherche en Nouvelle-Zélande a fourni beaucoup de connaissances fondamentales sur *D. geminata*, y compris la première analyse de la séquence de gène 18S rARN de *D. geminata* et la découverte de la capacité de cette diatomée à survivre à l'extérieur des habitats aquatiques, notamment dans les semelles de feutre des cuissardes utilisées par les pêcheurs.

Deux ans plus tard, en 2006, l'apparition de proliférations de *D. geminata* dans les rivières à saumon de l'est du Québec a soulevé l'inquiétude dans cette région en raison des dommages potentiels sur les populations de saumon Atlantique et des effets d'ordre écologique et esthétique. L'Atelier international sur *Didymosphenia geminata* a fourni une tribune pour un groupe d'experts scientifiques du monde entier afin qu'ils puissent présenter aux parties intéressées du Québec, des provinces et des États américains voisins des exposés sur :

1. la distribution, la détection, la génomique, l'écologie physiologique et les facteurs environnementaux favorisant les proliférations de *D. geminata*;

2. les effets potentiels des proliférations sur les populations de salmonidés;

3. les approches de gestion et de contrôle disponibles pour les agences gouvernementales;

4. des recommandations de recherches à effectuer pour améliorer la compréhension des proliférations et cibler les interventions des agences.

La coïncidence de l'Atelier de travail avec le Symposium internationale de limnologie (SIL) théorique et appliquée 2007 était très appropriée, puisque le Ministère du Développement durable, de l'Environnement et des Parcs (MDDEP) du Québec venait de confirmer la réoccurrence de didymo dans la Rivière Matapédia et l'apparition de cellules de didymo dans six rivières adjacentes de la péninsule gaspésienne (Isabelle Simard et Marc Simoneau, MDDEP). De plus, quelques semaines seulement avant l'Atelier, des proliférations de *D. geminata* avaient aussi été observées dans le Vermont, le New Hampshire et le nord de l'État de New York. Au moment de la réunion en août 2007, les gestionnaires des rivières du Québec et de plusieurs États américains avaient un urgent besoin d'information récente et cet intérêt s'est reflété dans la grande affluence observée à l'Atelier.

DISTRIBUTION, DÉTECTION, GÉNOMIQUE, ÉCOLOGIE PHYSIOLOGIQUE ET FACTEURS ENVIRONNEMENTAUX FAVORISANT LES PROLIFÉRATIONS

Le fait que la vaste répartition géographique de *Didymosphenia geminata* soit mieux documentée en Europe, en Asie et en Amérique du Nord suggère qu'elle origine probablement de ces régions. Avant 2004, les signalements de *D. geminata* dans l'Hémisphère sud étaient rares et pas toujours fiables (Kilroy 2007 ; Spaulding et Elwell, 2007). Bien que les proliférations de *D. geminata* en Nouvelle-Zélande aient recueilli une grande attention au cours des deux dernières années, des présentations de Brian Whitton (du Royaume-Uni) et d'Eli-Anne Lindstrøm (de la Norvège) ont rappelé aux participants de l'Atelier que *D. geminata* avait produit des proliférations dans quelques rivières de la Grande-Bretagne et de la Scandinavie pendant des décennies, sinon des siècles, ce qui signifie que les proliférations de *D. geminata* ne sont pas un phénomène nouveau dans le Vieux Monde.

Les proliférations de *Didymosphenia geminata* en Nouvelle-Zélande sont presque certainement le résultat d'une introduction d'espèce (Kilroy et al., 2004). La dispersion rapide de *D. geminata* dans les bassins versants de l'Île du Sud et l'ampleur des accumulations de biomasse dans certaines rivières caractérisent la biologie d'une espèce envahissante introduite pour la première fois dans un habitat favorable. Cependant, les causes des proliférations de *D. geminata* ailleurs dans le monde sont plus problématiques puisqu'elle est considérée indigène dans plusieurs de ces régions. *D. geminata* semble étendre sa répartition dans de nouvelles régions géographiques et de nouveaux types d'habitat. Les proliférations de *D. geminata* se produisent maintenant à des latitudes inférieures et dans des milieux d'eau plus chaude qui étaient auparavant considérés défavorables pour cet organisme (Spaulding et Elwell, 2007; Kumar et al., 2008). On ignore si ce changement est associé à l'introduction d'une nouvelle souche de l'espèce. Cependant, dans les régions où *D. geminata* est indigène, le patron de dispersion des proliférations entre les

bassins versants documenté dans la dernière décennie, soit l'Île de Vancouver (Bothwell et al., 2006), l'Islande (Jonsson et al., 2000), le Dakota du Sud, le Colorado (Spaulding et Elwell, 2007), suggère le mouvement envahissant.

Une des plus grandes priorités de Biosecurity New Zealand pour le programme *D. geminata* était le développement d'un outil moléculaire sensible et précis pour détecter la présence de *D. geminata* dans les bassins. Un tel outil a été développé par le laboratoire de Craig Cary à l'Université de Waikato et l'Université du Delaware. Le laboratoire Cary a analysé la séquence de gène 18S rARN de *D. geminata* et conçu plusieurs amorces PCR (réaction en chaîne par polymérase) spécifiques à didymo qui permettent de la distinguer parmi plusieurs espèces étroitement liées (Cary et al., 2007 ; les Comptes rendus de l'Atelier). Un protocole de réaction quantitative en chaîne par polymérase en temps réel (QRT-PCR) qui permet la quantification rapide de *D. geminata* dans des échantillons d'eau de rivières a été développé (Cary et al., 2007). La méthode est sensible, précise et robuste et est maintenant utilisée de façon routinière en Nouvelle-Zélande pour détecter les intrusions. La même analyse a été utilisée avec succès sur une gamme d'échantillons internationaux. Cette approche de dépistage de *D. geminata* en rivière, appliquée aux grandes régions géographiques, pourrait aider à identifier les facteurs qui déterminent sa répartition.

Cary et al. (2007 ; les Comptes rendus de l'Atelier) ont aussi commencé à explorer l'utilisation de marqueurs moléculaires dans les régions de l'espaceur transcrit interne (ITS) du gène 18S rARN pour distinguer les populations de *D. geminata* de régions géographiques différentes. Les découvertes préliminaires indiquent une séparation marquée entre les souches européenne et Nord-américaine de *D. geminata*. De plus, les travaux indiquent une proche filiation des populations de la Nouvelle-Zélande et de l'Amérique du Nord. Des travaux additionnels en cours utilisent les marqueurs d'ADN polymorphes amplifiés aléatoirement (RAPDs) et les répétitions de la séquence inter-simple (ISSR) pour établir la variation à une échelle plus fine. Le laboratoire de Cary, en accord avec Biosecurity New Zealand, a publié une demande mondiale pour obtenir des échantillons de *D. geminata* qui seront soumis à l'analyse génomique afin d'effectuer une étude phylogéographique de *D. geminata*. Les participants à l'Atelier ont été pressés de participer et des documents décrivant les procédures à suivre pour prélever, manipuler et soumettre des échantillons ont été rendus disponibles.

Plusieurs présentations à l'Atelier ont discuté les facteurs environnementaux associés à la croissance abondante de *D. geminata* dans les rivières et les attributs physiologiques qui pourraient favoriser son succès dans la compétition avec les autres espèces d'algues. La stabilité du débit et la faible turbidité ont été réaffirmées comme des facteurs importants contribuant à l'abondance *D. geminata* dans les rivières d'Alberta (Kirkwood et al., 2007 ; les Comptes rendus de l'Atelier). Ces deux conditions qui caractérisent les exutoires des lacs et des réservoirs aident à expliquer pourquoi ces environnements soutiennent souvent une biomasse élevée de *D. geminata*.

On a longtemps considéré *D. geminata* comme une espèce d'eau froide, mais des observations récentes dans des rivières plus chaudes (au moins pendant l'été) ont questionné cette hypothèse (Bothwell et al., 2006 ; Spaulding et Elwell, 2007). Une découverte intrigante de la présentation de Cathy Kilroy était que, peu importe la région dans le monde, *D. geminata* n'est jamais observée dans des cours d'eau ou rivières où la température minimale moyenne de l'air durant le mois le plus froid de l'année est supérieure à 5°C. L'implication que *D. geminata* aurait, durant

au moins une partie de l'année, une certaine exigence physiologique pour de l'eau froide afin de pouvoir prospérer est complètement nouvelle et appelle clairement à l'examen plus détaillé des données mondiales et la mise à l'épreuve expérimentale (Kilroy et al.., 2007).

La chimie de l'eau exerce aussi un fort contrôle sur la présence et l'abondance de *D. geminata*. Des observations effectuées en Norvège montrent que *D. geminata* exige des concentrations minimales de Ca (> 2 mg/l) et de S (> 2,5 mg/l). Les gradients de Ca et de S expliquent en grande partie le patron général de la répartition spatiale de *D. geminata* en Norvège (Lindstrøm et Skulberg, 2007 ; les Comptes rendus de l'Atelier). Les observations faites dans des cours d'eau de la Sierra Nevada corroborent cette découverte à l'effet que les concentrations plus élevées de Ca et de S permettent de distinguer les cours d'eau avec didymo et les cours d'eau sans didymo (Rost et al. 2007).

D. geminata a historiquement été associée aux eaux naturelles non polluées, c'est-à-dire pauvres en éléments nutritifs, mais des observations faites en Norvège et en Alberta indiquent que les degrés modérés d'enrichissement des cours d'eau en P inorganique peuvent stimuler sa croissance (Lindstrøm et Skulberg, les Comptes rendus de l'Atelier; Bowman, les Comptes rendus de l'Atelier). La phosphatase alcaline, une enzyme cellulaire superficielle qui permet aux cellules d'utiliser le phosphore organique dissous (POD) extracellulaire, s'est révélée localisée dans les tiges récemment sécrétées (Ellwood et Whitton, 2007). Cette enzyme pourrait fournir un avantage compétitif à didymo dans des environnements pauvres en éléments nutritifs où la majorité du phosphore dissous est présent sous la forme d'esters de mono - et de diphosphate. Il a été suggéré que les proliférations récentes de *D. geminata* dans certaines régions pourraient être associées à concentrations élevées de POD liées au changement des conditions climatiques (Whitton et Ellwood, les Comptes rendus de l'Atelier). On devrait considérer les concentrations de POD des rivières lorsqu'on évalue les facteurs environnementaux qui contrôlent l'abondance de didymo. En dépit de cette observation, *D. geminata* demeure une espèce sensible à la pollution dont l'abondance diminue quand le carbone organique total dépasse 6,5 mg/l et que le phosphore total (PT) excède 20 µg/l, du moins dans les eaux norvégiennes (Lindstrøm et Skulberg, les Comptes rendus de l'Atelier).

EFFETS POTENTIELS DES PROLIFÉRATIONS SUR LES PÊCHES DE SALMONIDÉS

Des informations critiques sur le statut et la productivité des populations de salmonidés des rivières historiquement touchées par *D. geminata* ont été présentées à l'Atelier. À cause de l'infestation récente de la rivière Matapédia, au Québec, l'impact potentiel sur le saumon Atlantique (*Salmo salar*) était d'une suprême importance pour beaucoup de participants. Lindstrøm et Skulberg (2007 ; les Comptes rendus de l'Atelier) ont démontré que des rivières norvégiennes étaient restées fortement productives pour le saumon Atlantique en dépit de proliférations de *D. geminata* qui remontaient à 150 ans en arrière. Certaines des rivières à saumon Atlantique les plus productives de Norvège ont aussi été le site des plus vastes proliférations de *D. geminata* dans ce pays (Lindstrøm et Skulberg, les Comptes rendus de l'Atelier).

Puisque les proliférations de *D. geminata* sont observées depuis plusieurs années dans les rivières scandinaves, il pourrait être argumenté que les effets sur le saumon Atlantique pourraient

être différents dans des régions récemment touchées par de telles manifestations. Cependant, l'évidence offerte par les récentes infestations de *D. geminata* en Islande soutient l'expérience norvégienne. Les proliférations de *D. geminata* qui ont débuté en 1994 dans les rivières de l'Islande n'ont eu aucun effet négatif évident sur les populations de *Salmo salar* (Jonsson et al., 2000; Jonsson et al., les Comptes rendus de l'Atelier).

Une analyse des vastes données historiques sur les retours et la productivité de trois espèces de salmonidés du Pacifique a été présentée lors de l'Atelier. Les populations de saumon coho (*Oncorhynchus kisutch*), de saumon chum (*O. keta*) et de truite brune (*O. mykiss*), élevées dans les rivières de l'Île de Vancouver touchées par des proliférations de *D. geminata*, ont été comparées aux populations des rivières sans *D. geminata* (Bothwell et al., les Comptes rendus de l'Atelier). Pour toutes les trois espèces, il n'y avait ou bien aucun impact significatif sur le retour et la productivité, ou il y avait un effet positif qui n'était pas cohérent pour toutes les rivières (Bothwell et al., les Comptes rendus de l'Atelier).

Les interactions entre les proliférations de *D. geminata* et le poisson sont dépendantes du cycle de vie de l'espèce, de ses habitudes alimentaires et de ses interactions avec d'autres espèces. Il n'y a aucun signalement d'effets négatifs de *D. geminata* sur les poissons anadromes, ces poissons qui passent seulement une partie de leur vie en eau douce. Nous pourrions demander s'il en est de même pour les espèces non anadromes, ces poissons qui passent tout leur cycle de vie dans les eaux intérieures. L'évidence présentée à l'Atelier concerne l'impact négatif fort de *D. geminata* sur la truite brune (*Salmo trutta*) résidente. Dans Rapid Creek (Dakota du Sud), un déclin abrupt de l'abondance des truites brunes adultes a coïncidé avec le commencement des proliférations de *D. geminata* en 2002 (Shearer et Erickson, 2006 ; Larson et Carreiro, les Comptes rendus de l'Atelier). Tandis que l'écroulement de la pêcherie de truite brune dans Rapid Creek, SD constitue une preuve tangible, l'évidence des effets de *D. geminata* sur la truite brune d'autres parties du monde manque toujours.

Une analyse des communautés d'invertébrés de Rapid Creek a suggéré qu'un goulot d'étranglement alimentaire puisse être responsable de l'abondance des « jeunes poissons de l'année (0-1+) » couplée avec l'absence d'adultes plus grands. Dans cette rivière, l'abondance de *D. geminata* est à l'origine d'un pourcentage inférieur de la communauté d'Éphéméroptères, Plécoptères et Trichoptères (EPT) qui est la plus importante pour les truites de plus grande taille (Larson et Carreiro, les Comptes rendus de l'Atelier). Un résultat semblable a été trouvé dans la Sierra Nevada lors de la comparaison d'un cours d'eau avec didymo et d'un cours d'eau sans didymo (Rost et al., les Comptes rendus de l'Atelier). Cette étude a aussi offert la preuve suggestive, par l'analyse d'isotope stable, que la truite dans un cours d'eau touché par *D. geminata* était moins dépendante du carbone algal que la truite d'un cours d'eau adjacent non touché par didymo (Rost et al., les Comptes rendus de l'Atelier). Des études de la composition des communautés benthiques effectuées en Nouvelle-Zélande ont trouvé des changements semblables dans le pourcentage des taxons EPT, mais sur une échelle absolue, l'abondance de tous les taxons d'insectes était toujours plus grande dans les secteurs de rivière touchés par des proliférations de *D. geminata* que dans les secteurs de rivière non touchés (Larned et al., 2007). Les réseaux trophiques, de par leur nature, sont complexes et interactifs. Les effets de *D. geminata* sur la dynamique trophique des cours d'eau devraient vraisemblablement varier en fonction de plusieurs facteurs, notamment l'étendue du recouvrement, l'épaisseur des amas et la

durée des proliférations. À cause de cette complexité, des contradictions entre des études différentes ne sont pas étonnantes.

APPROCHES DE GESTION ET DE CONTRÔLE DISPONIBLES POUR LES AGENCES GOUVERNEMENTALES

Prévention

Biosecurity New Zealand a passé en revue des options pour le confinement de *D. geminata* peu après sa découverte dans l'Île du Sud. L'augmentation du degré de conscience publique a été identifiée comme étant l'action la plus importante en se basant sur le raisonnement suivant : 1) l'extermination ou le contrôle d'une algue microscopique n'est pas vraisemblable à court terme, 2) les cellules de *D. geminata* vont probablement être disséminées au-delà des voies navigables connues touchées, 3) la restriction de l'accès des usagers aux rivières n'empêchera pas le risque de dispersion par les oiseaux et les autres animaux, 4) des méthodes de nettoyage appropriées pour les équipements utilisés en rivière ont été développées, 5) la sensibilisation du public va probablement permettre de changer davantage les comportements des usagers que les règlements et les fermetures.

Contrôle

L'analyse de dix agents potentiels de contrôle (algicides/biocides) a été amorcée par des essais expérimentaux (Jellyman et al., 2006). Les agents de contrôle ont été évalués sur la base de leur efficacité à causer la mortalité cellulaire et la dégradation de la biomasse. De plus, on a considéré les effets et les risques pour l'écosystème, la faisabilité d'application, le coût et la durée pour obtenir un contrôle efficace. Une deuxième phase d'expérimentation a été basée sur les résultats de la première étude. Des essais dans des canaux artificiels ont montré qu'un composé chelaté de cuivre (Gemex™) était le plus efficace dans l'élimination des cellules de *D. geminata* et la minimisation des effets sur les espèces non ciblées (Clearwater et al., 2007a). Finalement, une expérience de contrôle et d'extermination a été effectuée dans un cours d'eau touché (Clearwater et al., 2007b). Les résultats ont indiqué que le traitement pourrait être efficace dans la réduction, mais non dans la suppression de la population de *D. geminata*. Un certain nombre de ruisseaux issus de la nappe phréatique (résurgence), qui alimentent des rivières touchées par *D. geminata*, se sont révélés libres de toute population de didymo malgré leur exposition à la colonisation (Sutherland et al., 2007). Cette étude qui visait la comparaison de la survie de *D. geminata* sur des substrats artificiels déposés dans des rivières alimentées par le ruissellement de surface (pluies) et des ruisseaux issus de résurgences a révélé que la survie des cellules était faible dans les ruisseaux issus de résurgences. Bien que les facteurs ou la combinaison de facteurs influençant la survie n'aient pas été identifiés, ce type d'étude devrait être poursuivi dans le futur.

Monitoring et surveillance

Les outils qui utilisent l'empreinte génétique pour détecter la présence de *D. geminata* dans des bassins versants ont été développés et mis en oeuvre en Nouvelle-Zélande (Cary et al., 2007). La technique inclut des procédures de terrain pour le prélèvement efficace de cellules microscopiques et l'identification d'une espèce – la séquence d'ADN spécifique qui permet la différentiation de *D. geminata* parmi d'autres espèces de diatomées en Nouvelle-Zélande et dans les rivières internationales. En outre, des marqueurs génétiques provisoires ont été déterminés

qui indiquent que les cellules de *D. geminata* de la Nouvelle-Zélande ont probablement été importées de l'Amérique du Nord.

Éducation publique

Au cours des deux dernières années, beaucoup d'agences gouvernementales et non gouvernementales ont publié des renseignements sur *D. geminata* et formulé des recommandations pour le nettoyage des articles de pêche et de sports nautiques afin d'empêcher la dispersion de *D. geminata* par négligence.

BioSecurity New Zealand
http://www.biosecurity.govt.nz/didymo

Québec MDDEP
http://www.mddep.gouv.qc.ca/biodiversite/eae/didymo.htm

New Brunswick
http://www.gnb.ca/0254/FAQDidymo-e.asp

US Environmental Protection Agency
http://www.epa.gov/region8/water/didymosphenia/

NEANS
http://www.invasivespeciesinfo.gov/index.shtml

RECOMMANDATIONS POUR LA RECHERCHE ET LES INTERVENTIONS DES AGENCES GOUVERNEMENTALES

Les grandes proliférations de *Didymosphenia geminata* sont préoccupantes pour les écosystèmes fluviaux et des études demeurent nécessaires pour répondre à certaines questions fondamentales et résoudre des problèmes de gestion appliquée.

1) Il existe un patron récurrent dans l'apparition des proliférations envahissantes en Amérique du Nord, à commencer par celles de l'Île de Vancouver, Colombie-Britannique en 1989. Avec chaque signalement successif au Canada (Alberta, Yukon, Québec) et aux États-Unis (le Colorado, le Montana, le Missouri, l'Arkansas, le New Hampshire, la Pennsylvanie, le Vermont, New York), les gestionnaires font face au même problème d'information limitée sur les facteurs biologiques et environnementaux qui déclenchent la formation des proliférations. L'étendue temporelle et spatiale des amas pourrait être cartographiée en relation avec les activités anthropiques. De telles données pourraient permettre d'étudier l'interaction complexe des facteurs hydrologique, chimique, climatique et humain dans le développement des proliférations.

2) Les travaux préliminaires sur la génétique des populations suggèrent que les organismes de la Nouvelle-Zélande proviennent de l'ouest de l'Amérique du Nord, mais on ignore toutefois si la forme envahissante de *D. geminata* représente une souche nouvelle ou unique. Des marqueurs

moléculaires pour les populations de *D. geminata* doivent être développés davantage pour a) déterminer les liens entre les populations à travers le globe, et b) établir s'il existe une forme génétique unique responsable du comportement envahissant. Un effort en vue de recueillir des échantillons lors de réunions internationales récentes (ASLO, février 2007; SIL Août 2007) a été amorcé par Biosecurity New Zealand et nécessitera davantage d'appui.

3) L'analyse de l'abondance historique de *D. geminata* est possible grâce aux échantillons archivés existants et aux données de sédiments de lacs. Des échantillons de diatomées qui ont déjà été prélevés, traités et archivés peuvent être rendus disponibles pour l'analyse spécifique de *D. geminata*. C'est-à-dire que des lamelles peuvent être examinées pour étudier l'abondance de *D. geminata*. Dans la plupart des études, l'abondance des diatomées est basée sur les nombres totaux de cellules de diatomées et elle est largement influencée par la présence des petites cellules qui dominent la plupart des énumérations. Une telle approche est directe et peu coûteuse à accomplir. Une deuxième méthode pour étudier l'abondance historique consiste à identifier des rivières, touchées par des proliférations envahissantes, qui se déversent dans des lacs ou des réservoirs, déposant ainsi les cellules siliceuses dans des sédiments lacustres. Dans ces sites, les sédiments superficiels et l'abondance des cellules peuvent être corrélés pour reconstruire l'abondance historique du bassin de drainage.

4) L'abondance de *D. geminata* est liée aux effets sur la chaîne trophique, c'est-à-dire qu'avec une abondance plus grande, il y a des changements plus importants dans les communautés d'algues et d'invertébrés. Plusieurs études ont documenté les changements dans les communautés d'algues et d'invertébrés, cependant on ne connaît pas les implications quantitatives et à long terme. De façon particulière, les communautés d'algues et d'invertébrés affichent une plus faible diversité lorsque l'abondance de *D. geminata est* élevée que lorsque qu'elle est absente.

5) En dépit de l'importance et des effets de cet organisme, plusieurs aspects fondamentaux de sa biologie et de son cycle de vie demeurent inconnus. Bien que la reproduction sexuée ait été rapportée dans la documentation, elle n'a pas été observée dans les proliférations récentes. La division cellulaire végétative chez les diatomées résulte en des générations successivement plus petites de cellules et une partie cruciale du cycle de vie des diatomées est la restauration de la taille cellulaire maximale par la formation d'auxospores. L'étude de la distribution des tailles de cellules d'une population dans le temps pourrait fournir des connaissances fondamentales sur l'espèce, même si certains stades sexuels s'avèrent difficiles à observer.

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WORKSHOP AGENDA
August 18, 2007

Morning Session

8:00-8:15	Max Bothwell, Environment Canada
	Welcome
8:15-8:30	Sarah Spaulding, USGS/US EPA
	Basic diatom biology and ecology
8:30-9:00	Marc Simoneau & Isabelle Simard, MDDEP
	Status of didymo in Québec
09:00-09:15	Questions & Discussion

State of the Science, Part I - genomics/cell biology/autecology

0915-9:45	Craig Cary, Univ. Waikato/Univ. Delaware
	Didymo genomics, use in detection and phylogeographic studies
9:45-10:00	Questions & Discussion
10:00-10:15	Break
10:15-11:15	Brian Whitton, UK
	Didymo in the British Isles/Europe, hypothesis for bloom formation
11:15-12:15	Cathy Kilroy, NZ-NIWA
	Didymo in New Zealand, results of recent studies
12:15-12:30	Questions & Discussion
12:30-1:30	Buffet lunch for registered workshop participants

Afternoon Session

1:30-2:00 Mike Gretz, Michigan Technological Univ.
Chemistry and structure of didymo stalks

State of the Science, Part II Habitat characteristics of didymo

2:00-2:30 Andrea Kirkwood, Univ. Calgary
Factors controlling didymo biomass in Canadian Rocky Mountain Rivers

2:30-3:00 Andrew Rost, Univ. Nevada
Studies on didymo in Sierra Nevada streams

3:00-3:15 Questions & Discussion

3:15-3:30 Break

3:30-4:00 Sarah Spaulding, USGS/US EPA
Didymo in the continental United States and worldwide

4:00-4:30 Michelle Bowman, Utah State Univ.
Didymo response to low-level nutrient enrichment

4:30-4:45 Questions & Discussion

Dinner (Workshop participants make their own arrangements)

WORKSHOP AGENDA

August 19, 2007, Morning Session

State of Science, Part II - Impacts on Fish Ecology and Fisheries

8:00-8:30	Eli-Anne Lindstrøm, Norway
	History of didymo in Norwegian rivers and Atlantic salmon
8:30-9:00	Ingi Rúnar Jónssen, Iceland
	History of didymo in Icelandic rivers and Atlantic salmon
9:00-9:15	Questions & Discussion
9:15-9:45	Aaron Larson, South Dakota, USA
	Didymo impacts on Rapid Creek, SD and brown trout
9:45-10:15	Max Bothwell, Environment Canada
	Didymo impacts on steelhead trout and Pacific salmon
10:15-10:30	Questions & Discussion
10:30-10:45	Break

Management approaches to didymo

10:45-11:15	Christina Vieglais, Biosecurity New Zealand
	The New Zealand experience with didymo
11:15-11:45	Panel Discussion of management options
	Max Bothwell (moderator), Christina Vieglais (NZ), Sarah Spaulding (USA), Yves de Lafontaine (Can), Marc Simoneau (Quebec) and Fred Whoriskey (AFS)
11:45-1:15	Lunch (participants on their own)

Afternoon Session

Collaborative research efforts on didymo

1:15-1:45	Canadian research efforts on didymo
	Joseph Culp & Yves de Lafontaine, co-leads

1:45-2:15	International collaborative studies on genetic and morphological relationships of didymo populations Craig Cary & Sarah Spaulding, co-leads
2:15-2:30	Wrap up and comments.

Didymosphenia geminata Workshop

Abstracts for Presentations

On August 18, 2007

DIDYMO DANS LES RIVIÈRES DU QUÉBEC: ÉTAT DE SITUATION

Isabelle Simard[1] et Marc Simoneau[2]

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RÉSUMÉ

Les premières proliférations de l'algue *Didymosphenia geminata* (didymo) au Québec ont été observées à l'été 2006 dans la rivière Matapédia, une importante rivière à saumon du Bas-Saint-Laurent. En novembre 2006, des cellules de didymo ont aussi été détectées dans les rivières Patapédia, Humqui, Nouvelle, Cascapédia, Petite Cascapédia et Bonaventure, situées dans les régions du Bas Saint-Laurent et de la Gaspésie.

En 2007, des proliférations de didymo sont réapparues dans la rivière Matapédia et ont été observées pour la première fois dans les rivières Patapédia, Humqui, Nouvelle, Cascapédia, et Petite Cascapédia. Bien que les proliférations de la rivière Matapédia aient été notées sur une plus longue distance en 2007 qu'en 2006, elles étaient cependant de plus faible importance et limitées aux secteurs de la rivière où la profondeur de l'eau était inférieure à 1 mètre. Les proliférations qui ont touché les autres rivières étaient plus légères et elles ont été rapportées plus tard vers la fin de l'été ou au début de l'automne. Par ailleurs, des cellules de didymo ont aussi été détectées dans la Grande Rivière, mais n'ont pas entraîné de proliférations dans ce cours d'eau. Les niveaux d'eau plus élevés observés dans les rivières en 2007 pourraient avoir limité l'importance des proliférations. Toutes ces rivières feront l'objet d'un suivi au cours des prochaines années.

Jusqu'à présent, didymo n'a pas été détecté dans les régions du Saguenay et de la Côte-Nord qui comptent plusieurs rivières à saumon. On croit pour l'instant que les caractéristiques physico-chimiques de l'eau de ces rivières pourraient ne pas être aussi favorables à l'implantation de didymo que celles des rivières des régions du Bas-Saint-Laurent et de la Gaspésie.

En réaction à cette nouvelle problématique environnementale au Québec, un plan d'action gouvernemental a été élaboré par le ministère du Développement Durable, de l'Environnement et des Parcs (MDDEP) et le ministère des Ressources Naturelles et de la Faune (MRNF) en concertation avec les principaux partenaires régionaux, notamment les comités de bassin versant de rivières et les gestionnaires des rivières à saumon. Ce plan aborde les aspects de sensibilisation des clientèles, de prévention et d'acquisition de connaissances.

Divers documents ont été préparés pour informer et sensibiliser le public quant à l'importance d'adopter des mesures pour prévenir l'introduction et limiter la dispersion de l'algue didymo, ou de toute autre espèce indésirable, dans les rivières du Québec. Ces documents sont disponibles en français et en anglais sur le site Web du MDDEP à l'adresse suivante : http://www.mddep.gouv.qc.ca/biodiversite/eae/didymo.htm. Ils sont inspirés de l'approche INSPECTEZ, NETTOYEZ ET SÉCHEZ préconisée par Biosecurity New Zealand. Le principal message véhiculé est que tout usager d'un cours d'eau devrait agir comme si des cellules de didymo étaient présentes dans la rivière qu'il vient de quitter et absentes de la prochaine rivière qu'il s'apprête à visiter. En conséquence, pour éviter de disperser didymo, ou toute autre espèce aquatique envahissante, chacun devrait inspecter les pièces d'équipement qui sont entrées en contact avec l'eau, enlever tout amas d'algues et nettoyer son équipement avec l'une ou l'autre des solutions recommandées, ou le laisser sécher complètement jusqu'à ce qu'il soit sec au toucher, puis attendre 48 heures de plus avant de le réutiliser dans un autre plan d'eau.

RÉFÉRENCE

Comité scientifique MDDEP-MRNF sur l'algue *Didymosphenia geminata*, 2007. *Qu'est-ce que l'algue « Didymo » et comment prévenir sa propagation dans nos rivières?*, 3e édition, Québec, ministère du Développement durable, de l'Environnement et des Parcs et ministère des Ressources naturelles et de la Faune, ISBN : 978-2-550-49390-7 (PDF), 13 p. http://www.mddep.gouv.qc.ca/eau/eco_aqua/didymo/didymo.pdf

ABSTRACT

The first *Didymosphenia geminata* (didymo) bloom to occur in Québec was reported in the summer of 2006 in the Matapédia River, a major Atlantic salmon fishing river in the Lower St. Lawrence region. In November 2006, didymo cells were also detected in six other rivers (Matane, Sainte-Anne, Nouvelle, Grande Cascapédia, Petite Cascapédia and Bonaventure) located in the Lower St. Lawrence and Gaspésie regions.

In 2007, didymo blooms reappeared in the Matapédia River and were observed for the first time in the Patapédia, Humqui, Nouvelle, Cascapédia, and Petite Cascapédia Rivers. Even though the blooms observed in the Matapédia River covered a longer distance in 2007 than in 2006, their extent was less severe and they were restricted to areas where water was less than 1-meter deep. The blooms that affected the other rivers were light and occurred later at the end of summer or by early fall. Moreover, didymo cells were also detected in the La Grande Rivière but they did not translate into blooms. It is hypothesized that higher water levels observed during summer of 2007 may have limited the severity of the blooms. All these rivers will receive special attention in the years to come.

So far, didymo has not been detected in the Côte-Nord region despite the fact that it has many salmon rivers. At this point, it is hypothesized that the physical and chemical characteristics of water in the Côte-Nord rivers are not as favourable for didymo as those of the Bas-Saint-Laurent and Gaspésie rivers.As a result of this new environmental issue in Quebec, an action plan was

developed by the ministère du Développement Durable, de l'Environnement et des Parcs As a result of this new environmental issue in Quebec, an action plan was developed by the ministère du Développement Durable, de l'Environnement et des Parcs (MDDEP) and the ministère des Ressources Naturelles et de la Faune (MRNF) in close cooperation with regional stakeholders, namely the watershed and salmon rivers management organizations. The plan covers the aspects of user/stakeholder awareness, prevention and acquisition of knowledge.

Different documents were produced to inform and increase public awareness to the importance of adopting measures for preventing the introduction or for limiting the spread of didymo, or any other nuisance aquatic species, to other Québec rivers. These documents are available in French and English on the MDDEP Web site using the following hyperlink: http://www.mddep.gouv.qc.ca/biodiversite/eae/didymo-en.htm. They are based on the CHECK, CLEAN, and DRY campaign of Biosecurity New Zealand. The principal message carried out is that any river user should consider that the river he just visited contains didymo cells and the next one he is about to visit does not. So, in order to stop the spread of didymo and prevent the introduction of other aquatic invasive species, everyone should check any piece of equipment that came into contact with water. Users should remove clumps of algae, clean the equipment with recommended solutions, let it dry completely to the touch, inside and outside, then wait at least another 48 hours before using it again in another river.

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A SENSITIVE GENETIC-BASED DETECTION AND ENUMERATION METHOD FOR *DIDYMOSPHENIA GEMINATA*

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INTRODUCTION

The freshwater invasive benthic diatom *Didymosphenia geminata* is emerging globally as an organism with an extraordinary capacity to disrupt stream ecosystem function by forming persistent blooms of dense mucilaginous mats that can extend for several kilometers. The most common method for identifying *D. geminata* in water bodies involves the collection of algal scrapings and drift net-filtered water samples, their transport to a laboratory, and the identification of *D. geminata* frustules under a microscope. However, microscopy lacks sensitivity at low cell concentrations, especially in samples containing extensive detrital material and with the existence of morphologically similar diatoms. It is also a time consuming, labour intensive, and subjective method that restricts our ability to sample a wide range of rivers and sites within each river. Developments in molecular technology now allow rapid and specific low-level detection and enumeration of algal species (Handy et al. 2005, Coyne and Cary 2006, Rueckert et al. 2007). These techniques involve diagnostic gene amplification technologies that provide greater sensitivity and specificity of detection and enumeration of algal species with higher throughput when compared to microscopy, and are ideally suited for routine monitoring of field samples.

Here we report the development of a sensitive DNA-based protocol capable of detecting *D. geminata* in environmental samples with extreme sensitivity and specificity. Our specific requirements for this new methodology were that the method has 1) robust field capabilities from collection to quantification, 2) species or strain level specificity that has been environmentally validated, 3) extreme sensitivity for low-level detection (<1 cell ml^{-1}), 4) a broad dynamic range of detection (> 5 orders of magnitude), 5) an efficient, cost-effective, rapid, high-throughput laboratory capability, and 6) international application.

THE DNA METHOD

The DNA method relies upon amplifying a diagnostic gene (18S ribosomal RNA) from a field sample using the polymerase chain reaction (PCR). The target gene from didymo is initially fully sequenced and then, through comparison with other closely related diatoms, diagnostic regions of the gene are identified. PCR requires that two regions of the gene that discriminate

didymo from related taxa be used to generate didymo-specific primers that allow this region of the gene to be selectively amplified from a mixed community field sample. A variant of classical PCR is the quantitative PCR (QPCR) Taqman assay that incorporates the use of a third didymo-specific fluorescent element (a probe) that allows the original amplification to be reported with extreme sensitivity using a laser light source and CCD sensor-based instrument (Figure 1). The QPCR method can determine didymo cell concentrations to a much finer resolution than microscopy when the samples have low cell numbers (single cell ml^{-1}) or contain large amounts of sediment or organic matter.

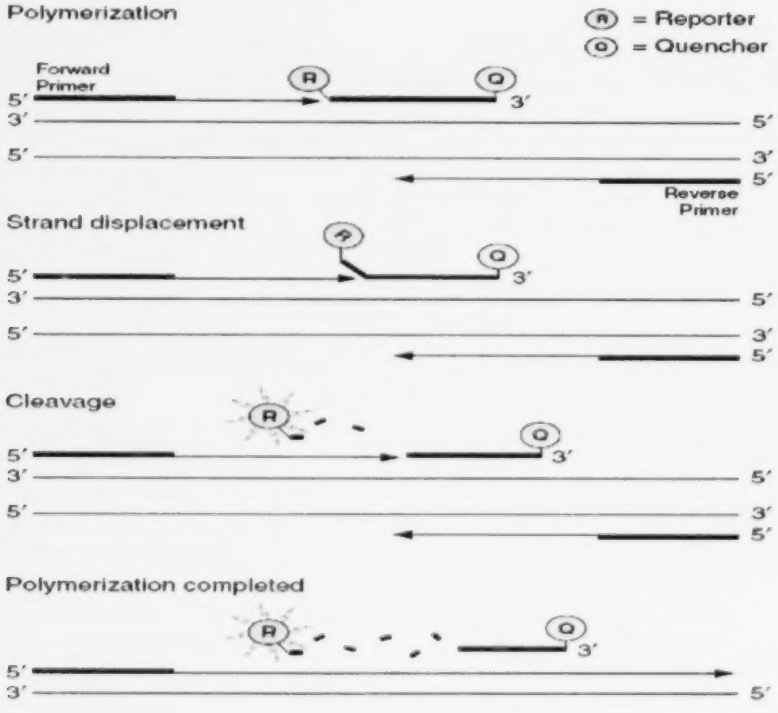

Figure 1. Principle of the quantitative PCR Taqman method. Source: Applied Biosystems, Inc.

The QPCR assay designed for *D. geminata* (Cary et al., 2007) proved to be both sensitive and robust, with detection of approximately 30 copies (~0.1-1 cell) of the 18S ribosomal RNA gene from *D. geminata,* and rarely produced false positive amplification signals. The method was tested with environmental samples and found to be extremely sensitive, in some cases detecting *D. geminata* in samples where microscopy had reported a negative. From these analyses we have set the lower end of detection (sensitivity threshold) to be 1 pg of our calibrator sample (Figure 2), which is equivalent to ~1.0-0.1 *D. geminata* cells in the QPCR Taqman reaction.

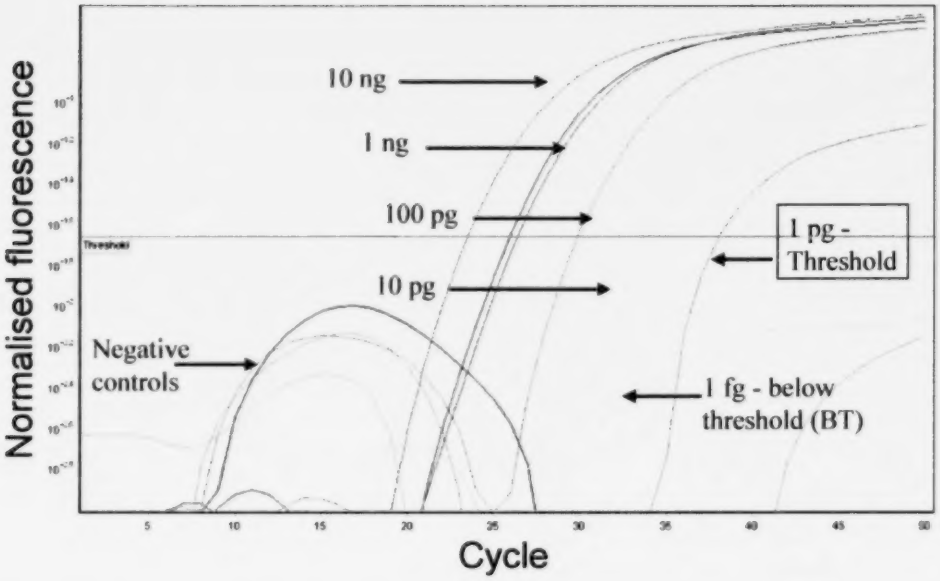

Figure 2. Taqman profiles of calibrator sample (40B) at 10 X dilutions identifying the 1 pg threshold of detection.

Although the Taqman QPCR method appears to be extremely sensitive and specific to *D. geminata*, the specificity of the method to detect only *D. geminata* in New Zealand waters required extensive validation. The primers and probe therefore underwent a robust three-tiered validation procedure consisting of 149 positive QPCR in which amplification products were validated by gel electrophoresis (PCR amplicon length), high resolution melt (HRM) analysis (heat denaturation characteristics), and direct DNA sequencing of the QPCR product. We initially examined 174 samples from 76 rivers by QPCR, 54 from the South Island, 8 from the North Island, and 12 international (Norway - 4, Canada - 2, England - 1, and USA - 5). 149 samples were positive for *D. geminata* (i.e., above threshold; Fig. 2). All of these positive samples have been validated to be *D. geminata* using all three validation procedures. No North Island samples were positive for *D. geminata*. These results indicate that the Taqman QPCR method is specific for *D. geminata*.

The DNA method has now been adopted as the primary monitoring tool for surveillance in the North Island of New Zealand where, to date, didymo has not been detected. The efficacy of the method lies in its highly sensitive negative predictive value and allows us to maintain high frequency surveillance over a large geographic area. The QPCR Taqman assay also allows the ability to enumerate the number of cells present in a given sample, which could be useful for measuring the effectiveness of mitigation efforts.

PHYLOGEOGRAPHY

In addition to developing a DNA-based surveillance method, a secondary objective was to leverage the molecular information acquired while developing the DNA surveillance method towards a phylogeographic study to shed light on the possible origins of the *D. geminata* population in New Zealand and internationally. Preliminary results indicate that the partial 18S+ITS region provides a sufficient level of resolution to reveal the phylogeographic history and origin(s) of *D. geminata* in New Zealand. From the limited number of samples that have been analysed, it appears more likely that the New Zealand *D. geminata* originated from an incursion from North America rather than Europe.

ACKNOWLEDGEMENTS

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GROWTH AND NUTRIENT ECOLOGY OF *DIDYMOSPHENIA* IN BRITISH ISLES AND OTHER EUROPEAN COUNTRIES

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SUMMARY

An outline is given of the seasonal growth cycle of *Didymosphenia geminata* in R. Coquet, a small upland river in northern England where there is a long record of the species being abundant. Although it is most conspicuous some distance downstream of the source, the diatom was also found in a small tributary near the source, emphasizing the importance of studying the whole catchment. A detailed study of its growth at another fast-flowing site showed that the water chemistry was characterized by high N:P and high organic:inorganic P. In view of this and the facts that the diatom showed high phosphomonoesterase and phosphodiesterase activities and (at least) the former was located in the stalks it is suggested that success of the species is favoured by organic phosphate in the environment. This fits with fragmentary data on chemistry and detailed observations on other algae and their surface phosphatase activities at sites elsewhere in the British Isles with *Didymosphenia*. Control measures should be based on this assumption. These include managing the catchment to minimize release of organic P and the deliberate addition of inorganic P to enhance the inorganic:organic P ratio. It is recommended that this is done in spring at the most upland site where *D. geminata* would otherwise form dense growths.

This account is based on surveys and experimental studies in northern England, together with records from other parts of the British Isles, Poland and Russia. The observations in Poland were made jointly with Prof. Barbara Kawecka (Institute of Nature Conservation, Kraków) and those from Russia were communicated to the authors by Dr. Sergey Komulaynen (Karelian Research Centre).

UK records for *Didymosphenia geminata* started in the mid-19th century and it is unclear to what extent increases have occurred in recent years. Mass growths have been recorded in a stretch of R. Coquet, Northumberland, since (at least) the late 1950s, though growths were sparse in 2007. The catchment consists mainly of grassland on peaty soils overlying base-rich rocks. *D. geminata* has been found in a small tributary near the source and then in a long stretch of the main river. It is unclear how much the population downstream persists there all year or depends on re-inoculation from upstream. *D. geminata* occurs as motile cells on rocks in winter and then forms stalks in mid-spring. Cells removed from rocks in early spring are often highly motile, reaching 10μm s^{-1}. Although it seems likely that cell aggregation is a key initial step in colony formation, attempts to show this in the laboratory have been unsuccessful. The often rapid increase in colonies in early summer presumably depends on cells being released from larger colonies. However, cells detached at this time of year seldom show movement in the laboratory

and, if they do, the rates are less than cells from rocks in early spring. Auxospore formation was noted in a colony in the tributary near the source in early August 2007.

During a 2-yr study of water chemistry and nutrient ecology of aquatic mosses (Ellwood et al., 2008) in another upland stream in northern England, phosphatase assays were also conducted on *Didymosphenia* from March to August 2000. The stream drains peaty soils and limestone and the catchment is used for sheep grazing at low stock density. The main conclusions presented by Ellwood and Whitton (2007) are as follows. Although the water chemistry (measured twice a month) was highly variable, it was characterized by high N:P and with most of the filtrable phosphate in the organic fraction (Table 1). Organic phosphate averaged 85% of the filtrable phosphate, with a maximum concentration in April. Assays for surface phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities were conducted under standard conditions after return to the laboratory to show the potential for colonies to hydrolyze phosphate monoesters and diesters, respectively. PMEase activity was low in early March, but high for the rest of the period and especially so in June and July. Activities of PMEase and PDEase were mostly quite similar similar (Table 1). Use of BCIP-NBT (5-bromo-4-chloro-3-indolyl phosphate - nitroblue tetrazolium) staining procedure showed that PMEase activity occurred in the stalks (Fig. 1). A more detailed study of colony structure and staining with colonies from R. Coquet in June 2006 also showed marked PMEase activity, with staining in the upper part of the stalks and the cells remaining unstained. It is suggested that organic phosphates (monoesters and diesters) are hydrolyzed in the stalk and the resulting inorganic phosphate passes to the cell via the stalk.

Table 1. Summary of data concerning N and P in Stony Gill, N. Yorkshire, based on sampling twice a month from January to July 2000. Details are given for nutrients in 2-µm filtrable fraction of water and phosphatase activities of *D. geminata*. FRP, filtrable reactive P; TIN, total inorganic N; TFP, total filtrable P; FOP, filtrable organic P. Assays were done with *para*-nitrophenyl phosphate and bis-*para*-nitrophenyl phosphate and activity assessed by nitrophenol release.

	Min	Mean (SD)	Max
FRP (g L^{-1})	3.2	5.2 (3.6)	14.5
TIN (g L^{-1})	20	121.1 (83)	250
TIN : FRP (by mass)	2.1	30.9 (23.9)	70.2
FOP as % TFP	67.8	85.7 (9.0)	95.9
PMEase (µmol mg chl a^{-1} h^{-1})	2.7	12.4 (10.4)	34.5
PDEase (µmol mg chl a^{-1} h^{-1})	3.7	13.0 (7.3)	23.7

The facts that *Didymosphenia* occurred in an environment where it was potentially P-limited, that organic phosphate was the main form of filtrable ("soluble") phosphate and that it showed high phosphatase activities and this activity was localized in the upper part of the stalk together indicate the importance of organic phosphate for this diatom. We suggest that high N:P and high organic to inorganic phosphate ratios in the water are key environmental factors favouring *D. geminata* and this is supported by observations from other streams and rivers.

Figure 1. BCIP-NBT staining of phosphatase activity (PMEse) in the region of the stalk close to the cell.

The UK sites where the diatom is known to the authors all combine drainages from organic-rich soils and limestone or base-rich rocks and have relatively few houses in the catchment. At least under low-flow conditions, the water is moderately hard. However, it is unclear whether hardness favours *Didymosphenia* directly or is merely correlated with the type of catchment favouring release of organic P to streams, especially the high pulses in spring. None of the sites surveyed are regulated by dams and all undergo large, seasonally irregular, changes in flow, though none ever dry completely. Several rivers where *Didymosphenia* has sometimes been abundant in Poland (e.g. R. Dunajec, R. San) are downstream of dams, raising the possibility that flow regulation may be more important for the species under the hydrological (and perhaps chemical) conditions of a more continental type of climate than in the British Isles. The results of surveys in Karelia, North-West Russia, indicate that *Didymosphenia* may be more widespread here than in any other region of Europe reported so far.

The study raises a number of practical matters for management. The failure of water management organizations, including those in the UK and Ireland, to include organic phosphate in surveys is a hindrance to interpreting the wider literature. This means that general surveys on diatoms already made on rivers of some European countries are of limited use for understanding the ecology of *Didymosphenia*. The following are possible approaches to controlling nuisance growths.

1. Catchment management should aim to minimize changes likely to enhance the organic phosphate concentration of the water. Possible factors which might do so include deforestation and soil disturbance, especially on peaty or other organic-rich soils, and deliberate release to the river of liquids or other materials with a high organic content.

2. Understanding of the distribution of the diatom within the catchment is needed to avoid the risk of repeat re-inoculation from upstream.

3. As it seems likely that *Didymosphenia* (and perhaps other stalked diatoms) compete(s) for organic P more effectively than most non-stalked diatoms, enhancing inorganic:organic P should favour non-stalked diatoms. This should be done in early spring at the time when *Didymosphenia* is just starting to form stalks. Once the colonies have started to form, it is probably be too late to achieve much success in that particular year. It might be necessary to continue phosphate addition for several months each year. The site chosen for phosphate addition should be towards the upper end of the distribution range within the river, though it may be impractical to include small tributaries. Experimental studies are needed to establish the ratio of phosphate forms needed for non-stalked diatoms to outcompete *Didymosphenia*, but inorganic P would probably need to be well in excess of organic P. Phosphate addition would be most effective, if the inflow were matched to the river flow.

4. It might be possible to enhance the competitive success of algae less dependent on organic phosphate by using agents selectively inhibitory to PMEase and PDEase. For instance, Durrieu et al. (2003) showed that the heavy metals Cr, Ni, Cu, Zn, Cd, Hg and Pb were all highly inhibitory to *Chlorella vulgaris* surface PMEase activity. However, the present authors would discourage such an approach, because of the wider implications for the ecosystem. In addition, upland streams with elevated Zn and Cd are often dominated by metal-tolerant strains of other organic phosphate-utilizing algae (Whitton et al., 2005), so *Didymosphenia* might also evolve strains tolerant to these and perhaps other metals.

The authors suggest that the increases in *Didymosphenia* in Europe and elsewhere, sometimes reaching nuisance proportions, are probably the result of enhanced breakdown of peat or other organic-rich soils leading to an increased export of organic phosphate to drainage streams. Atmospheric N deposition, climatic warming and catchment disturbance are all known to be factors enhancing peat degradation. If climatic warming proves the most important, the effect on *D. geminata* may be due to an increased and extended period of high organic phosphate concentrations in spring.

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DIDYMOSPHENIA GEMINATA IN NEW ZEALAND: DISTRIBUTION, DISPERSAL AND ECOLOGY OF A NON-INDIGENOUS INVASIVE SPECIES

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The first verified identification in New Zealand of the stalked, freshwater diatom *Didymosphenia geminata* was in October 2004, after unusual algal mats were discovered in the Lower Waiau River, Southland, South Island (Kilroy 2004). The discovery led to a prompt biosecurity response because of reports of unsightly blooms of the species in Vancouver Island, Canada, in the 1980s, and more recent reports of blooms from central Europe (e.g., Kawecka and Sanecki 2003). Mean biomass of the blooms at some sites in the Lower Waiau River was four to 10 times higher than that recorded previously (Figure 1). The increase was particularly striking because, in this regulated river, high algal biomass was already considered to be a problem.

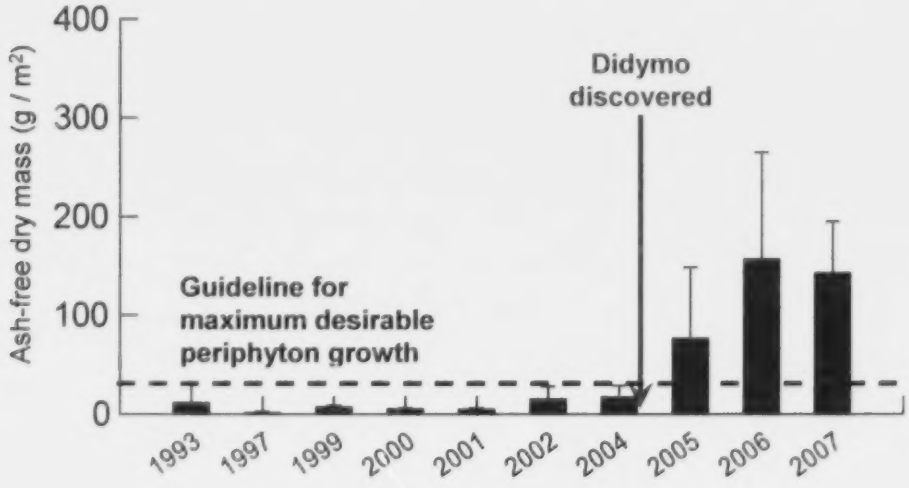

Figure 1. Periphyton biomass at a monitoring site in the lower Waiau River, Southland, New Zealand, 1997 to 2007.

At the time of the 2004 discovery, little formal information was available on the biology, ecology and potential impacts of *D. geminata* and a series of studies was initiated to address three broad questions: Where is *D. geminata* in New Zealand, in terms of geographic range and ecological niche? What are its effects? How can it be controlled? This summary covers selected results from these studies as at August 2007.

D. geminata was not reported from any other New Zealand river until September 2005, when it was discovered in several rivers in central and northern South Island. Since then the species has been detected in many other South Island rivers (Figure 2), but has still not been detected in the

North Island. The invasion was tracked in a sequence of delimiting surveys at up to 400 river sites throughout New Zealand. Sampling comprised a combination of benthic sampling (algal scrapes from multiple substrate particles) and water filtration through a 40-□m-mesh plankton net. These field methods were designed to maximise the chances of including *D. geminata* cells in a sample, if they were present at the site (Kilroy and Dale 2006).

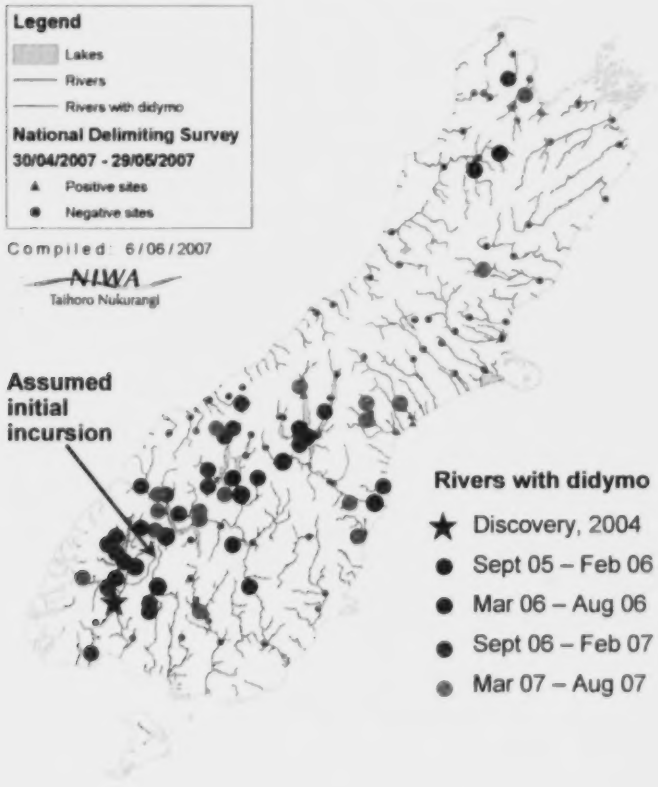

Figure 2. Sequence of spread of *D. geminata* throughout the South Island up until August 2007 (based on a map in Duncan 2007).

The pattern and speed of the spread of *D. geminata* through the South Island strongly suggests human-mediated dispersal. Almost all the positive finds have been in accessible river reaches with high recreation use (angling, kayaking and four-wheel driving), compared with few finds in less accessible rivers. *D. geminata* is not known to possess any resistant resting stage or spore that would facilitate its dispersal. However, laboratory tests showed that cells may remain viable for many weeks in cool, damp conditions (Figure 3). Felt-soled waders, used almost universally by anglers, were suspected of providing a good transfer medium because the material retains moisture for long periods. Preliminary trials showed that more live cells were retrieved from felt soles than other common materials used in river recreation (neoprene, rubber soles, leather boot upper) following overnight drying (Figure 4) (Kilroy et al. 2007a).

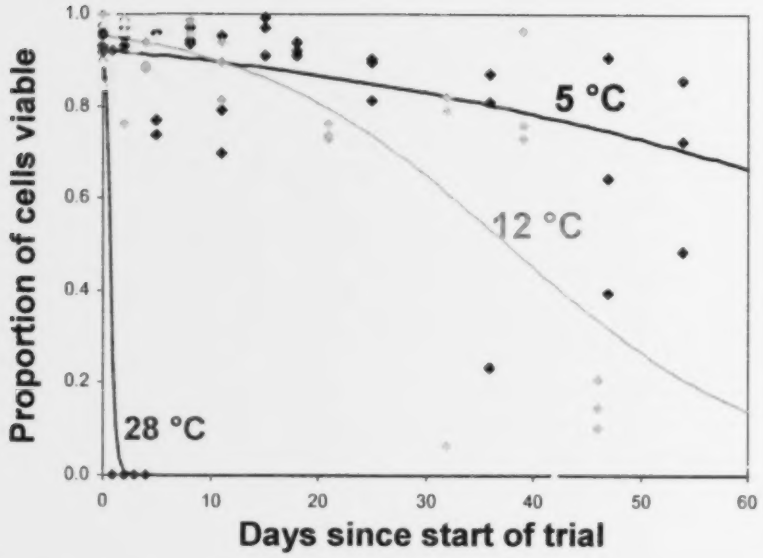

Figure 3. Viability of *D. geminata* at different temperatures. Colony pieces were maintained in river water in a 16:8 h light:dark regime at 5, 12, 20 and 28 °C, and periodically tested for cell viability using a Neutral Red staining technique (Kilroy et al. 2007a).

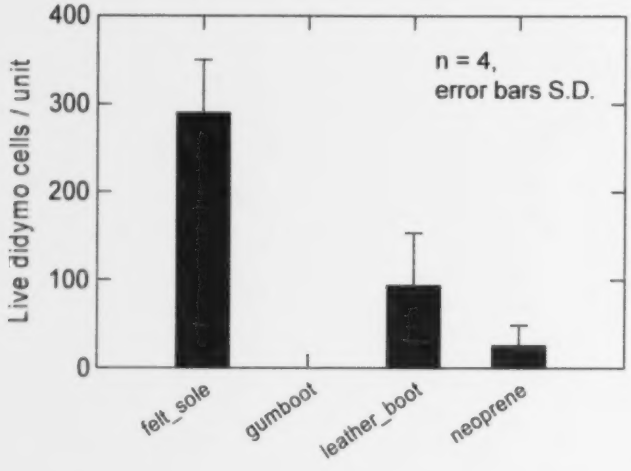

Figure 4. Mean numbers of live cells (with intact chloroplasts) detected per unit after scrubbing/rinsing four common materials worn by river recreationalists, following initial cleaning with water and overnight drying at 17 °C.

An initial attempt to determine the potential distribution of *D. geminata* in New Zealand, based on ecological suitability (i.e., not considering dispersal pathways), was undertaken within six months of the initial discovery. In the absence of known ecological preferences, the analysis was

based on an estimate of "ideal" ecological conditions for *D. geminata*, drawing on information from the literature and unpublished sources. The entire New Zealand river network was described using existing environmental information, and environmental distances from the ideal were calculated. The resulting "likely environments map" indicated that much of the South Island was highly susceptible, but the North Island was much less so. The difference was due mainly to an assumption that *D. geminata* is a cool-water species (Kilroy et al. 2008).

Once *D. geminata* became established in multiple rivers, there was opportunity to re-assess its potential distribution in New Zealand. In March–April 2007, surveys were undertaken at 150 sites in 17 affected rivers, involving visual estimates of *D. geminata* % cover and mat thickness, with collection of associated environmental data. Using this dataset along with national-scale environmental information, we derived models for *D. geminata*, which were used to run predictions of % cover and mat thickness in all New Zealand rivers (Kilroy et al. 2007b). In addition to providing more robust predictions for the occurrence of *D. geminata*, the survey and modelling also provided insights into the environmental drivers of the species. Our models indicated that lake influence was the most important predictor for both % cover and mat thickness, followed by substrate stability (described by substrate size and rock hardness variables). Time elapsed since a significant flood (defined as approximately 3 × median flow, based on previous correlative studies, Clausen and Biggs 1997) was also a significant predictor, confirming that bloom formation is more likely in rivers subject to long periods of low flows, which may include regulated flows. Absolute temperature was a minor component of both models, though seasonality (the difference between summer and winter temperatures) was positively correlated with both measures (Table 1). The model results were consistent with our observations during the survey that, in many rivers, mat thickness and extent declined in a downstream direction.

Table 1. Contribution of environmental variables to models explaining % cover and mat thickness of *D. geminata*, based on a survey of 150 sites in 17 affected rivers in South Island, New Zealand. For details of variables and units, refer to Kilroy et al. (2007b). Direction: +, positive relationship; –, negative relationship; nl, non-linear relationship.

	Model for % cover		Model for thickness	
Variable	%contrib.	direction	%contrib.	direction
Lake influence	32.3	+	29.0	+
Hard rock in catchment	15.1	+	5.6	+
Days since a flood	13.2	+	8.9	+
Bed substrate size	9.9	+	10.6	+
Temperature seasonality	6.7	+	9.8	+
Reach slope	5.7	–	5.3	–
Pastoral land in catchment	5.0	nl	4.2	–
Days with high rainfall	4.8	–	7.9	nl
Calcium	4.2	nl	3.6	–
Mean January temperature	3.2	–	4.6	–
Nitrate			5.3	–
Mountain influence			5.3	+
Model cross-validated R^2	0.52		0.48	

Despite the small contribution of temperature to the new models, our predictions still indicated that the North Island is very much less susceptible to *D. geminata* than the South Island. This effect was partly due to the fact that many North Island rivers lay outside the environmental envelope of the survey sites. Thus the predictions had reduced reliability. Global-scale patterns of *D. geminata* distribution with respect to temperature indicate that there may be an environmental upper limit of a mean air temperature in the coldest month of approximately 5 °C, which would exclude much of the North Island. However, without a clear physiological explanation, such a limit remains speculative.

The ecological effects of *D. geminata* blooms on New Zealand rivers are still unclear. High variability in correlations between aquatic invertebrate indices and *D. geminata* biomass preclude definite conclusions, though high biomass is certainly associated with dramatic increases in overall invertebrate densities, these increases comprising mainly dipterans and non-insect taxa (Kilroy et al. 2005; Larned et al. 2008).

There is now no prospect of eradicating *D. geminata* from the South Island. Current priorities are therefore to try to prevent the species reaching North Island rivers, to limit its spread in the South Island, to protect iconic and highly valued sites in the South Island, and to further elucidate the drivers of patterns of *D. geminata* distribution. The latter includes investigations into why some

spring-fed streams appear to be resistant to colonisation, a phenomenon that has been confirmed in field experiments, but which occurs at too fine a scale for inclusion in our national models and predictions.

ACKNOWLEDGEMENTS

Funding for this work was provided by Meridian Energy Ltd (Lower Waiau periphyton surveys, Figure 1) and MAF Biosecurity New Zealand (all other studies). The distribution map (Figure 2) is derived from surveys organised by Maurice Duncan (NIWA) for MAF Biosecurity New Zealand and was produced by Helen Roulston.

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THE STALKS OF DIDYMO

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Production of prodigious amounts of stalk material differentiates *Didymosphenia geminata* (didymo) from other benthic diatoms and is a very important aspect of its biology which contributes to nuisance blooms. If didymo was just another benthic diatom producing a uniform 1- 5 mm thick brown scum/slime on rocks, it would most likely not be the subject of discussion. Attempts at control or mitigation of didymo should include limiting stalk proliferation or eliminating stalk mass.

When didymo cells divide, the stalk bifurcates, the end result of which is an overall branched structure with stalks intercalating and coalescing to form an aggregate "woven fabric" mat that contains algae, macroinvertebrates, detritus and other stream debris. *D. geminata* stalks are composed primarily of sulfated polysaccharides with significant uronic acid content, and protein. Monosaccharide analysis has revealed predominately galactosyl and xylosyl residues and linkage analysis has showed predominately 3,4-Gal and 4-Xyl. The polysaccharide portion of the stalk therefore appears to be primarily sulfated xylogalactan, which has been reported for stalks of related diatoms *Gomphonema* and *Cymbella*, where it was shown to be intrinsically hydrophilic and linked by ionic cross-bridging.

Partial degradation of stalks with several chemical and enzymatic agents revealed that stalks consisted of concentric layers of material with differing chemical composition. The hydrated xyloglucan component of the stalk was surrounded by an outer striated layer which was resistant to degradation. All tested mechanical methods to degrade stalk mats were ineffective on hydrated mats although dry mats could be chopped. Chemical agents that degraded stalks included concentrated HCl and HNO_3, NaOCl and the chelator EDTA. EDTA and NaOCl were effective over long exposure periods. Treatment with acids required high concentrations and elevated temperatures to achieve significant stalk degradation, limiting effectiveness in natural environments. Enzymatic degradation of stalks was most effective and crude enzyme extracts from the fungi *Penicillium funiculosum*, and *Aspergillius niger*, as well as the enzyme based dietary supplement *Omega-zyme* showed significant degradation potential.

The success of enzymatic degradation of stalk masses has application in future mitigation and biocontrol initiatives. We are currently in the process of identifying specific inhibitors of stalk synthesis.

DIDYMOSPHENIA GEMINATA DISTRIBUTION AND BLOOM FORMATION ALONG THE SOUTH-EASTERN SLOPES OF THE CANADIAN ROCKIES

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Some of the earliest anecdotal reports of *Didymosphenia geminata* outbreaks along the eastern slopes of the Canadian Rockies occurred in the late 1990s, in Banff and Jasper National Parks. Our research group was able to document *D. geminata* distribution in 2004 and 2005, by assessing preserved periphyton samples collected from two main-stem headwater rivers (Bow and Red Deer) in southern Alberta. The Bow and Red Deer rivers exist in parallel sub-basins of the South Saskatchewan River Basin (SSRB), and share similar edaphic characteristics, but contrasting flow regimes and intensity/type of land use. The flow regimes and water quality contrasts between these otherwise comparable rivers was useful in elucidating why *D. geminata* was present in both rivers, but conspicuous and bloom-forming in the Bow River only. In particular, our results showed that the coefficient of variation in discharge and water turbidity were significantly different between the two rivers, and were also important in predicting the presence/absence of *D. geminata* (i.e. *D. geminata* presence was associated with lower water turbidity and coefficient of variation in discharge compared to sites where *D. geminata* was absent). We were also able to show a significant negative relationship (r^2=0.30) between *D. geminata* biomass and mean discharge, indicating that low discharge velocities were associated with bloom events (Figure 1).

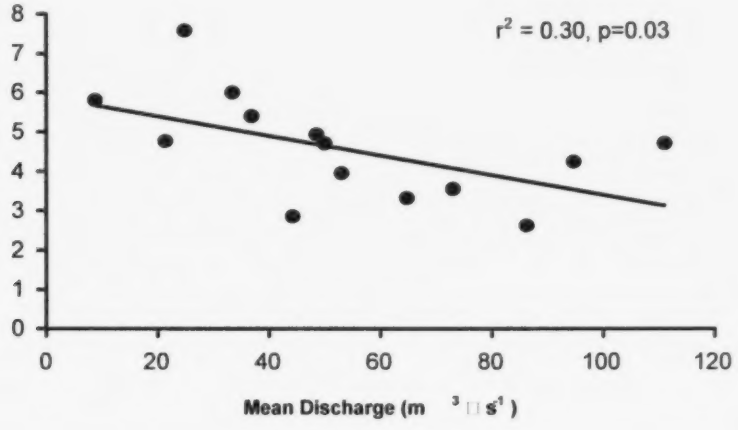

Figure 1. Relationship between the monthly mean discharge experienced by periphyton communities at the time of collection, and *D. geminata* abundance in the Bow and Red Deer rivers.

In 2005, we conducted experiments using nutrient-diffusing substrata (NDS) to determine if *D. geminata* responded to variations in nitrogen (ammonium nitrate) and phosphorus (phosphate) concentrations and combinations of the two. At one particular site in the Bow River, we deployed the NDS trays during a *D. geminata* bloom event (i.e. all cobble substrate was carpeted with *D. geminata*). The NDS trays and natural substrate samples were collected 2-weeks after the initial deployment. *D. geminata* marginally increased on natural substrate over the 2-week period, suggesting that the bloom was in stationary phase (embedded graph in Figure 2).

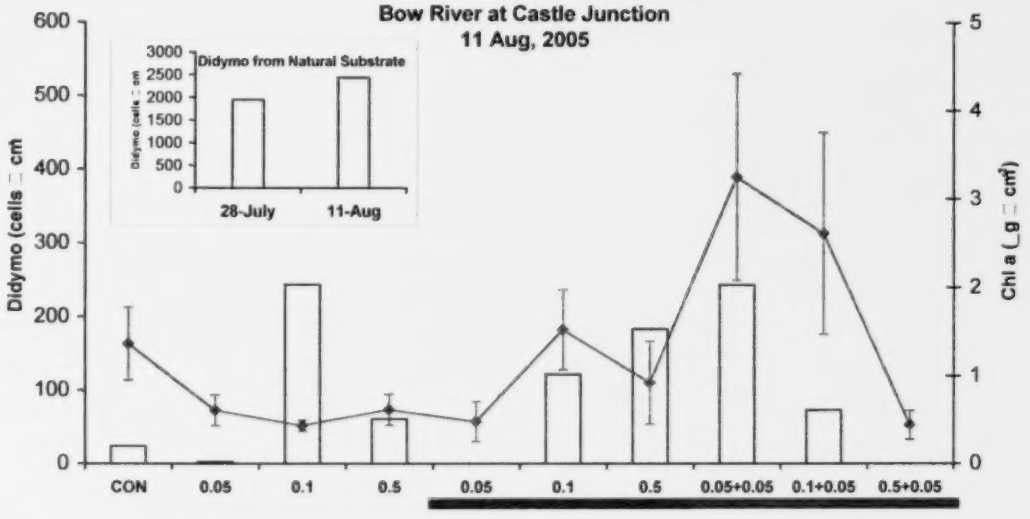

Figure 2. *D. geminata* abundance on different nutrient treatments after a 2-week deployment in the Bow River at Castle Junction (64-km from headwaters). Molar concentrations of nutrients are presented on the x-axis and reflect: ammonium nitrate (yellow bar), phosphate (blue bar), and combinations of ammonium nitrate + phosphate (red bar). Total chlorophyll a measured for each treatment is represented by the green line. Abundance of *D. geminata* on natural cobble substrate is presented for both the day of deployment (28 July) and day of retrieval (11 Aug) in the graphical insert.

Colonization and establishment of *D. geminata* on NDS treatments was highly variable, and indicated no obvious preference for the increased availability of inorganic nitrogen or phosphorus (Figure 2). Chlorophyll a values among treatments were also highly variable, and were not significantly different (ANOVA, $p>0.05$) from the control. What these data indicate is that there was a high degree of colonization heterogeneity, and that given a 2-week establishment and growth period, there was no obvious growth enhancement from any of the nutrient treatments for *D. geminata* or the algal community as a whole.

In 2006, we expanded our study to include 16 additional headwater rivers of the SSRB. From this expanded dataset we were able to determine that *D. geminata* could be detected in most rivers sampled, but only bloomed at certain sites, particularly those situated immediately below dam spillways. This was not only a spatial trend, but also remained consistent from year to year (although seasonality of blooms was somewhat variable). In the Red Deer River, for example, we were able to sample from 3 sites for three consecutive years (2 sites above Dickson Dam, and one site below). In every year sampled, *D. geminata* consistently had the highest cell densities below Dickson dam, compared to the two upstream sites. This spatial and temporal trend is further supporting evidence for the role of dams as hotspots for *D. geminata* outbreaks.

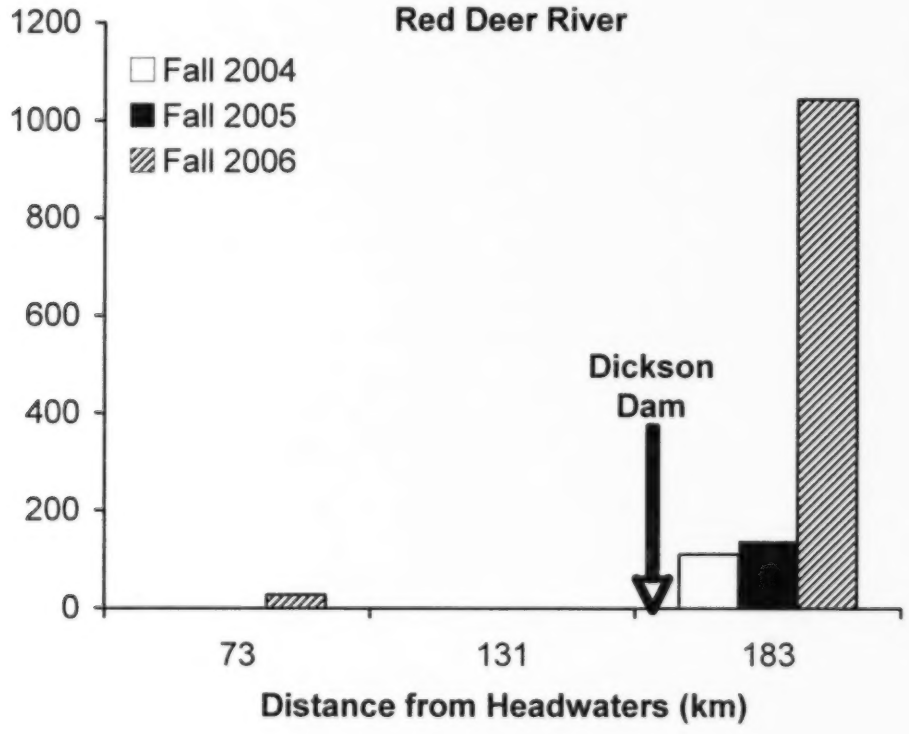

Figure 3. *D. geminata* abundance over three years at three sampling sites extending from the headwaters of the Red Deer river, to below the spillway of the first impoundment of the river system (Dickson Dam).

ENVIRONMENTAL CONTROLS AND POTENTIAL FOOD WEB IMPACTS OF *DIDYMOSPHENIA GEMINATA*, A COMPARATIVE ECOSYSTEM STUDY

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Similar stream reaches, (one with and one without *Didymosphenia geminata*) in the eastern Sierra Mountains of California were monitored in 2005 and 2006 to better determine the environmental controls and potential food web impacts of a stalked diatom bloom containing didymo. Data shows creeks are similar in geography, aspect, slope, wetted width, substrate, canopy cover, and nutrients (nitrite, nitrate and phosphate). The creeks differ in that the creek with didymo has reduced disturbance regime, EPT taxa, seasonal variation in the BMI and algal community composition and elevated ion chemistry (calcium, sulfate and silicate) and water temperature (2-3 C higher). Preliminary stable isotope data shows disparate carbon signatures between periphyton, grazers and predators in the didymo creek while carbon signatures in the non-didymo creek are similar between multiple trophic levels. From this comparative ecosystem study, a series of in-situ, mesocosm and in lab autoecological studies will begin in the spring of 2007 to further our understanding of the ecosystem controls and consequences of stalked diatom blooms dominated by didymo.

CONFIRMED DISTRIBUTION OF *DIDYMOSPHENIA GEMINATA* (LYNGBE) SCHMIDT IN NORTH AMERICA

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Observations indicate that the diatom *Didymosphenia geminata* (Lyngbe) Schmidt is expanding its geographic and ecological range in North America, however few historical voucher specimens exist to substantiate the apparent trend. We compiled confirmed distribution records of the current geographical and ecological range of *D. geminata.* Presence and absence records of the species were obtained from the EPA Environmental Monitoring and Assessment Program (EMAP), the USGS National Water Quality Assessment Program (NAWQA), and other sources (see Kumar et al. submitted). Over of 4,750 sites were evaluated for the presence of *D. geminata* and of those sites, the diatom was confirmed in 308. The sites covered fifteen states and three provinces in the United States and Canada (Fig. 1).

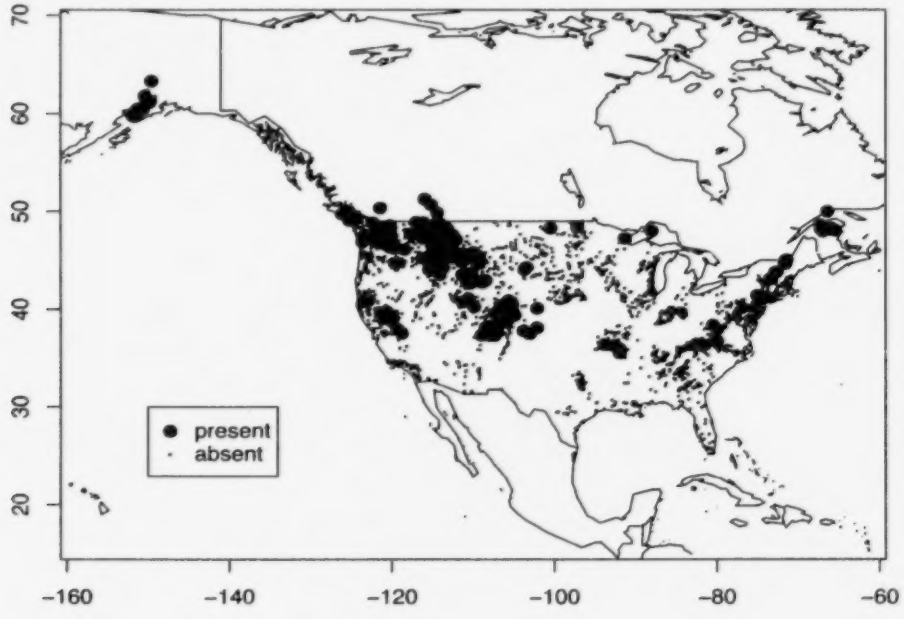

Figure 1. Presence and absence records (samples) of *D. geminata* in the United States. Over 4,750 samples were included, and *D. geminata* is present in 308 sites. Records are based on data from the USGS National Water Quality Assessment (NAWQA), the EPA Environmental Monitoring and Assessment Program (EMAP), and samples from other studies. Note that this map does not include samples and published records in Alaska and northern Canada.

Didymosphenia geminata is widespread in Asia, Europe, and North America. In North America, historical reports of *D. geminata* are primarily taxonomic or floristic (Cleve 1894-1896, Boyer 1916) and often lack information on abundance. A number of records are from Alaskan sites (Manguin 1960, Patrick and Freese 1961, Foged 1981). Others report *D. geminata* in the continental United States (Fox et al. 1967, Zingmark 1969, Nelson et al. 1973, Prescott and Dillard 1979, Stoermer 1980, Moffat 1994). Recent habitat models demonstrate that the potential distribution of *D. geminata* in the United States is based primarily on climatic variables (mean temperature of the warmest quarter and base flow index) (Kumar et al. submitted).

Preliminary calculations show that 4483 km (20% +/- 13%) of Colorado mountain streams and 17,660 km (6% +/- 2%) of western United States streams are estimated to contain *D. geminata*. These estimates are based on presence of *D. geminata* cells, rather than the abundance of cells and formation of nuisance blooms.

Although we do not have estimates of the distribution and extent of nuisance blooms, we expect the actual presence of *D. geminata* to be greater than our preliminary estimates. Standard protocols for measuring diatom abundance in stream assessment underestimate both presence and relative abundance of *D. geminata*. For example, in a comparison of approximately 800 microslides analyzed by EPA protocols (count of 300 cells, equal to 600 valves) with microslides analyzed by counting all *D. geminata* on the entire slide at low magnification, there was no relation between the two methods (Fig. 2). Furthermore, 15 of the samples were reported as lacking *D. geminata*, but up to 80 valves were present on the microslide. The discrepancy can be explained by the fact that *D. geminata* cells are large relative to the small diatoms and may not be encountered with the standard protocol. To quantify *D. geminata* abundance in confirmed blooms, we suggest using a macroscopic measure (Kilroy et al. 2008) to estimate coverage of *D. geminata* mats in streams.

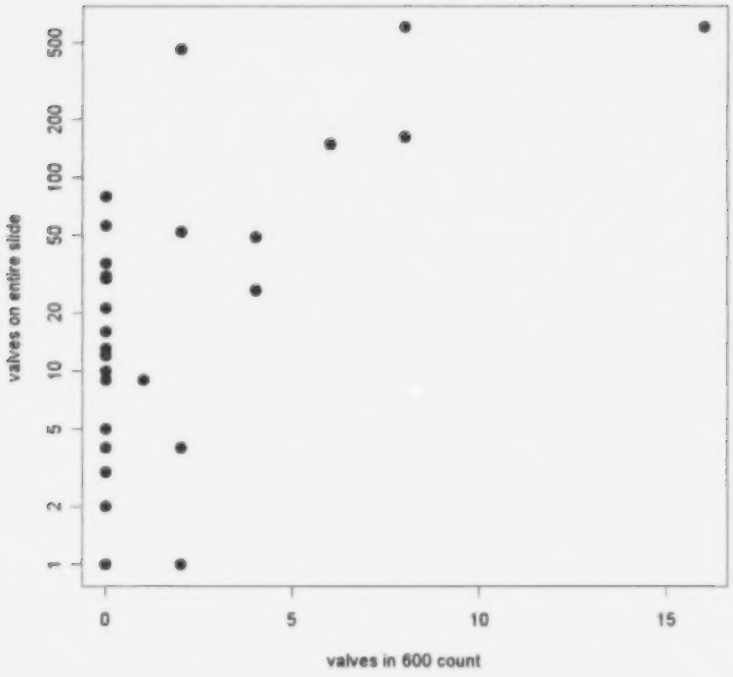

Figure 2. Comparison of the number of *D. geminata* valves reported in a 300 cell (600 valve) count from the EPA western EMAP project compared with the number of *D.* geminata valves counted (log 10 scale) on the entire microslide. This comparison shows that standard protocols for counting diatom cells can result in false negative reports.

CONCLUSIONS

The greatest number of reports of *D. geminata* are from the Rocky Mountains, Pacific Northwest, and Sierra Nevada Mountains in California. Reports of nuisance blooms from Missouri, Arkansas, Quebec, Vermont, and New Hampshire have occurred in recent years. Although we used existing surveys to estimate the presence of *D. geminata* in North America, we lack information on the temporal and spatial extent of nuisance blooms. It is important to note the distinct difference between microscopic presence of a few cells and macroscopic coverage of stream reaches by the mucilaginous stalks of this diatom. Because additional surveys could be logistically difficult and expensive to implement, we suggest that examining detailed controls on growth of *D. geminata* within a narrow region or watershed is most cost-effective.

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INCREASED *DIDYMOSPHENIA GEMINATA* BIOMASS IN RESPONSE TO LOW-LEVEL PHOSPHORUS ENRICHMENT OF OLIGOTROPHIC RIVERS

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Didymosphenia geminata or 'didymo' was found in both natural and unusually high abundances during surveys conducted in 1998-2007 to monitor nutrient enrichment of Rocky Mountain Rivers within the Canadian National Parks. Water and algal samples were collected in October both upstream and downstream of wastewater treatment plant discharges near the Lake Louise, Banff, and Jasper townsites. Each treatment plant was upgraded in the winter of 2002-3 to improve phosphorus removal from effluent; although phosphorus removal from Lake Louise effluent was relatively efficient prior to 2002.

Occurrences of unusually high *D. geminata* biomass were associated with low-level phosphorus enrichment of the Bow and Athabasca Rivers (Table 1). Bowman et al. (2005) showed that prior to treatment upgrades, epilithon at upstream sites was severely limited by the availability of phosphorus whereas there was little or no limitation in downstream sites. *D. geminata* biomass was consistently low at phosphorus-limited, upstream sites (i.e., < 60 $\mu g/ cm^2$) and consistently high at phosphorus-replete, downstream sites (i.e., > 60 $\mu g/ cm^2$, > 7000 $\mu g/ cm^2$ downstream of Lake Louise).

Table 1. Positive relationship between phosphorus availability and didymo abundance.

River	Location	Site	Years	Nutrient status of epilithon[1]	Mean (sd) of total phosphorus (µg/L)	Mean (sd) of didymo biomass[2] (µg/cm²)	Frequency of didymo detection (% years)
Bow							
	Lake Louise	Upstream	1998-2007	Severe P	2.0(0.9)	0	0
		Dowstream	1998-2001	Slight P	2.2(0.4)	0[3]	0[3]
			2002-2007	no data	2.7(1.3)	58672(26893)	100
	Banff	Upstream	1998-2007	Severe P	3(1)	6(23)	20
		Dowstream	1998-2002	P saturated	69(29)	210	20
			2003-2007	no data	21(27)	190(89)	100
Athabasca							
	Jasper	Upstream	1998-2007	Severe P	5.6(2.4)	39(27)	40
		Dowstream	1998-2002	P saturated	7.6(3.8)	308(345)	40
			2003-2007	no data	6.9(3.2)	2279(1781)	60

[1]Derived from alkaline phosphatase activity measured in 2000 (Bowman et al. 2005)

[2]Didymo biomass was estimated from cell counts and volumes

[3]Viable cells were found in spring and summer, not autumn but didymo stalks consisitently carpeted the riverbed in all seasons

Increased *D. geminata* biomass coincided with increased phosphorus availability within locations but phosphorus concentrations were not always indicative of the extent of bloom formation. Detection of viable cells downstream of Lake Louise in autumn coincided with increased phosphorus concentrations in 2002-2007 relative to 1998-2001. However, the site downstream of Lake Louise had the highest *D. geminata* biomasses (i.e., was consistently carpeted with *D. geminata* stalks throughout spring, summer, and autumn 1998-2007) but the lowest phosphorus bioavailability and phosphorus concentrations of the downstream sites studied (Table 1). In addition, although riverine phosphorus concentrations have declined in sites downstream of Banff and Jasper since treatment plant upgrades, *D. geminata* abundances have remained high and frequencies of detection have increased (Table 1).

Although *D. geminata* responded to low-level enrichment of these oligotrophic rivers, phosphorus availability alone could not account for the magnitude of blooms. Similarly, Kirkwood et al. (2007) found that while stable flow regime was important in determining the extent of *D. geminata* bloom formation in lower reaches of the Bow and Red Deer rivers, other environmental factors were also important. Bowman et al. (2007) concluded that abundance of epilithon in these sites was proximately limited by phosphorus availability only when light was not limiting and ultimately controlled by temperature and river discharge. A compilation of the chemical and physical habitat characteristics where *D. geminata* blooms form and their biological and hydrologic effects are needed to predict and manage *D. geminata* globally.

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Didymosphenia geminata Workshop

Abstracts for Presentations

on August 19, 2007

DIDYMOSPHENIA GEMINATA – A NATIVE DIATOM SPECIES OF NORWEGIAN RIVERS COEXISTING WITH THE ATLANTIC SALMON

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INTRODUCTION

Didymosphenia geminata, recognised by botanists in Norway for the last 150 years, has a wide regional Scandinavian distribution. A detailed analysis of the distribution and growth of *Didymosphenia* in Norwegian rivers is in preparation (Lindstrøm and Skulberg 2008). For more than five decades the Norwegian Institute for Water Research (NIVA) has conducted research on benthic algae in Norwegian watercourses. Most studies included measurements of chemical and physical conditions as well as the biota. Benthic algae data have been processed into a database that can be linked to water chemistry and river habitat characteristics. We have analysed relations between these datasets to acquire knowledge about the distribution, the water chemistry requirements and the river habitat preferences of *Didymosphenia* in Norway (Lindstrøm & Skulberg 2008). In this presentation we also comment on the relationship between profuse *Didymosphenia* growth and the status of Atlantic salmon (*Salmo salar)* fisheries in some rivers.

Water quality

Observations in Norwegian rivers show that D*idymosphenia* has a stronghold in northern and middle Norway, but is apparently absent from the south-western part of the country. This is mainly attributed to the natural quality of inland waters being a key factor regulating the distribution of *Didymosphenia* in Norway. The south-western part of Norway has wide areas with hard inert bedrock (mainly granite and gneiss), and the water is correspondingly nutrient poor with low ionic content and little buffering capacity (Ca generally <1 mg/L and pH <6.5). *Didymosphenia* appears to be excluded from such water, and has never been observed in water with calcium concentrations <1.8 mg/L or pH <6.7. Similarly, *Didymosphenia* seems to depend on some modest level of organic enrichment to thrive, and only water with total organic carbon >2.0 mg C/L supports *Didymosphenia* proliferations. The diatom is pollution sensitive and tends to disappear when total organic carbon exceeds 6.5 mg C/L and total phosphorus 20 µg P/L. *Didymosphenia* is also sensitive to heavy metals, and proliferations were never observed in water with copper concentrations >10 µg/L or zinc correspondingly >25 µg/L. Our data suggest that elevated sulphate levels (>2.5 mg/L) is a prerequisite for *Didymosphenia* to proliferate.

River habitat preferences

Didymopshenia is found in the four biomes in Norway including Svalbard (an archipelago in the Arctic Ocean under Norwegian administration): arctic, alpine, boreal and boreonemoral. It grows in water ranging from 0° to 23°C, but is more common and develops higher biomass in cool water that seldom exceeds 18°C in the vegetative period. *Didymosphenia* thrives in arctic regions characterized by a long period of darkness alternating with a period of constant daylight on an annual basis (e.g. Svalbard), and in regions that have boreal diurnal light-dark alternation. The local light conditions are important, and *Didymosphenia* appears to depend on an unshaded habitat to thrive. Preferred river habitats in Norway are shallow riffles with coarse stable substratum and a steady flow regime. Stream regulation resulting in reduced flooding and more constant flow tend to promote the build up of *Didymosphenia*.. *Didymopshenia* displays remarkable permanence in rivers. It has been frequently observed developing large populations over the past 40 to 140 years in a number of localities (Lindstrøm & Skulberg 2008) and is expected to prevail in these rivers in the future, assuming no major change in environmental conditions.

Profuse growth of *Didymosphenia* in rivers with Atlantic salmon

Almost a century ago Schmidt-Nielsen and Printz (1915) reported profuse growths of *Didymosphenia* from River Dramselva in April 1911. Similar proliferations have been observed repeatedly in a wide range of watercourses in Norway and seem to be a naturally occurring phenomenon (Skulberg 1984) (Figure 1). Proliferations occur from April to October, in regulated as well as unregulated rivers. What these rivers have in common is naturally nutrient rich water with relatively high calcium content (>4 mg Ca/L) and pH (>7.0). What actually triggers the proliferations is only partly understood. Incidents of flooding followed by low steady flow during some weeks seem to foster profuse growths. Several rivers with *Didymosphenia* proliferations are known for their high production of Atlantic salmon. River Tana frequently has the highest catch of Atlantic salmon in Norway. *Didymosphenia* was first recorded in this river in 1868 and it is still common there. Atlantic salmon is present in 4 of the 8 rivers shown in Table 1, and they are among the best rivers for Atlantic salmon in Norway (Hansen et al. 2007).

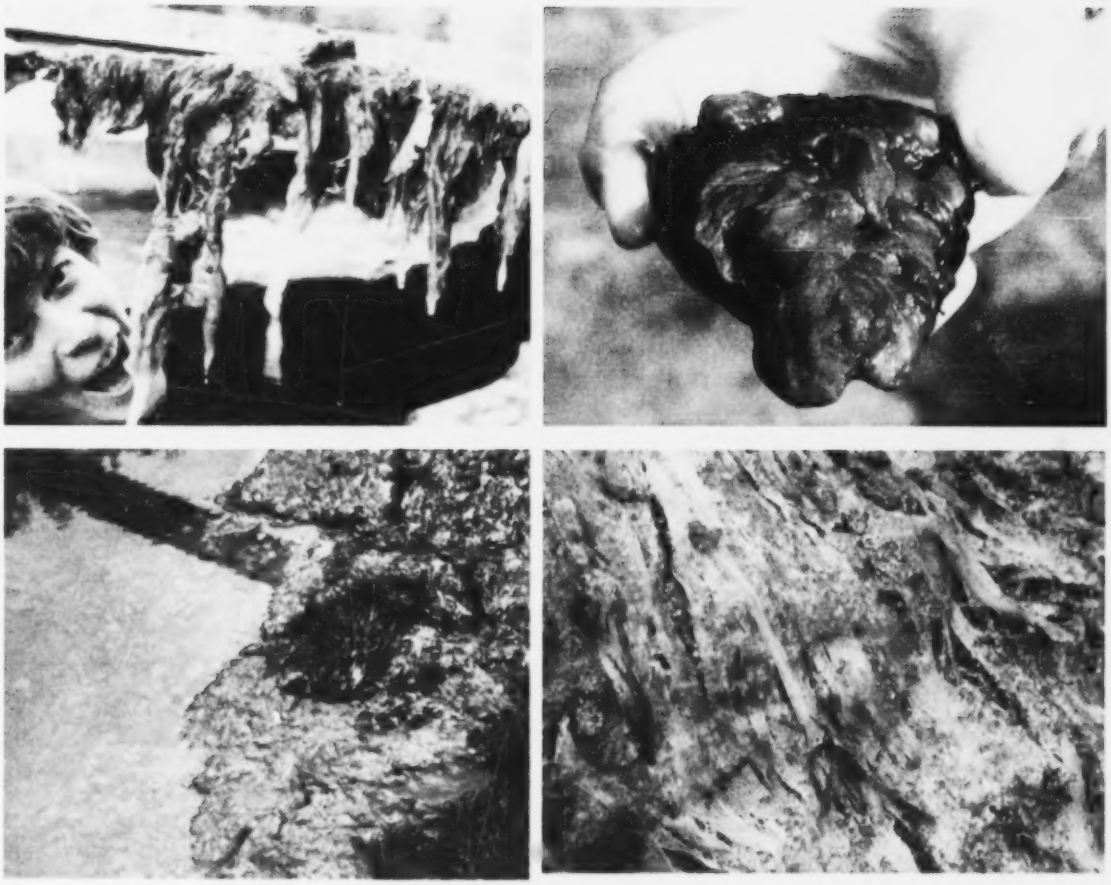

Figure 1. Profuse growth of *Didymosphenia geminata.* A wooden stick collected downstream the outlet of a lake in River Andselva (Aug. 1983) was completely infested with *Didymosphenia* and so was the riverbed (upper left). The other pictures are from River Nidelva (July 2007) where 3-5 cm thick mats (upper right) covered the riverbed (lower left), and formed a paper like film of stalks drying on newly emerged river shores (lower right).

Table 1. Norwegian Rivers with profuse growth* of *Didymosphenia geminata*.

Locality	Date of observation	Status of Atlantic salmon
River Tana North Norway	Sept. 1989	Frequently the highest catch in Norway (46.1 metric tons in 2006)
River Alta North Norway	Oct. 1981	One of the best rivers for fishing in Norway, had the fourth largest catch in 2006 (20.5 metric tons)
River Andselva (outl. L. Andsvatn) North Norway	Aug. 1983	No anadromous fish (but brown trout)
River Orkla Mid Norway	Sept./Oct. 1980-2000	Well known river for its fine stock, the third best catch in Norway in 2006 (23 metric tons)
River Nidelva Mid Norway	Aug. 1964 July 2007	Known for its stock of large salmon, catch in 2006 was 4.5 metric tons
River Dokka South Norway	June 2006	No anadromous fish (but brown trout)
River Sagelva South Norway	Aug. 1997	No anadromous fish, status otherwise unknown
River Dramselva (at Vikersund) South Norway	April 1911	No anadromous fish (but brown trout)

* *Didymosphenia* covers >50% of the riverbed

Status of Atlantic salmon in Norway

The catch of Atlantic salmon reported by the countries located around the North Atlantic Ocean has decreased dramatically since 1980, and is now only 20 percent of what was reported then (ICES 2007) (Figure 2). The reduction is also severe in Norway, from 1,800 metric tons in 1980 to 900 in 2006 (Hansen et al. 2007). Norway accounted for 46 percent of the total catch during 2006 and has therefore a major responsibility for the management of Atlantic salmon today.

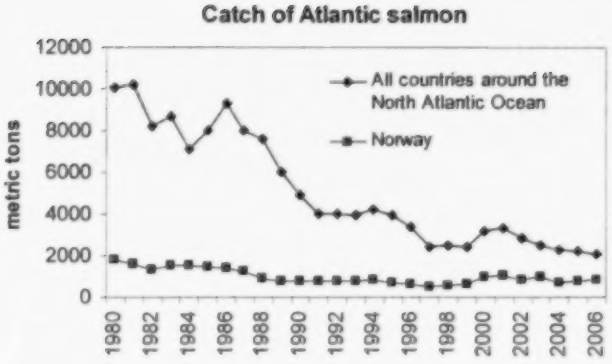

Figure 2. Annual catch of Atlantic salmon – Norwegian share (Hansen et al. 2007) and total catch in all countries around the North Atlantic Ocean (ICES 2007).

There are at least 6 major causes for reduced catch of Atlantic salmon in Norway (Hansen et al. 2007).

- *Regulated flow* – the single factor that has caused the greatest loss of salmon in rivers (the main reason in 19 of the 45 rivers where salmon is reported lost).
- *Pollution* - from agriculture, sewage and industrial wastes.
- *Gyrodactylus salaris* – this digenean ectoparasite was introduced to Norway on salmon parr imported from Sweden in 1975. It infects young fish in the river and causes increased death rate and reduced smolt production. Some of the infected rivers have been treated with rotenone to kill the parasite, but the treatment has only partly been successful.
- *Acid rain* – during the last century deposition of sulphate and other acidifying components into water bodies with low buffering capacity has extensively caused harmful high aluminium levels in lakes and rivers in Norway. One of the consequences of acidification and elevated aluminium has been the depletion or eradication of salmonids and other fish in these watercourses. Today acidification problems are reduced and the water quality is improving slowly. Liming is carried out in several rivers to counteract the negative effects of acid rain. River Mandalselva was a good river for salmon fishing before 1970, however later on the salmon was almost eliminated due to acidification. The river has been limed since 1996. After recovery it has been among the 10 best rivers for salmon fishing. During 2005 and 2006 the total catch of Atlantic salmon has been 11 and 12 metric tons, respectively.
- *Aquaculture* - is an increasing worry. The high incidence of salmon lice (*Lepeophtheirus salmonis*) on the farmed salmon is presumed to infect and kill outgoing smolt, and escaped farmed salmon interbreed with wild salmon resulting in reduced "fitness" for a life in the river.
- *Over-fishing* – legislation has been implemented to counteract over-fishing. Driftnet fishing in the fjords was prohibited in 1989. Regulations have also reduced the use of gillnets and keyways in the rivers.

Although *Didymopshenia* was never reported to impact the Atlantic salmon negatively, it has been reported to foul fishnets and other fishing gear from time to time.

We have no long-term serial data of the presence and development of *Didymosphenia* populations in relation to the catch of Atlantic salmon in Norwegian rivers. Further, we have limited knowledge of the ecological interactions between *Didymosphenia* and the other river biota, i.e. salmon parr and the organisms they feed on nor of potential interactions between *Didymosphenia* and the returning salmon during spawning and migration. It is, however, not likely that profuse developments of *Didymosphenia* will effect the salmon spawning or the egg development, since spawning takes place in late fall and winter, outside the period of maximum *Didymosphenia* biomass. Also, the preferred *Didymosphenia* substratum is typically solid rocky bottom which differs from the coarse gravel where the salmon lay their eggs.

Theoretically, *Didymosphenia* may have both positive and negative effects on salmon survival and growth. The diatom community may provide support and nutrition for the benthic food web that salmon parr depend upon and thus promote better smolt yields in *Didymosphenia* rivers. Such ecological interactions are important questions for future field studies and experimental investigations.

ACKNOWLEDGEMENTS

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OCCURRENCE AND COLONIZATION PATTERN OF *DIDYMOSPHENIA GEMINATA* IN ICELANDIC STREAMS

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The diatom *Didymosphenia geminata* was first reported in Icelandic streams in 1994, when an unusual amount of algal growth was reported in the River Hvítá in the Borgarfjörður area in West Iceland (Figures 1, 2). According to local residents the algal growth was first visible in 1992 and increased the following years. It caused a nuisance to net-fisheries, as the stalks clogged the gill-nets. The algal growth was of great concern, particularly because many of the most valuable recreation fishing streams in Iceland are located in the area (Jónsson et al. 1997). Salmon and trout fisheries are of high economic value in Iceland. The economic importance of salmon and trout fishing rights varies depending on region, but contributes as much as 50% of the total income for the fishing right owners in productive salmon areas, such as in west Iceland. A survey conducted on streams in West Iceland in 1994 revealed that the diatom occurred in several of the tributaries of the River Hvítá (Figure 2).

Figure 1. A dense mat of *Didymosphenia geminata* in the River Norðurá in West Iceland in 1994.

Following the initial discovery in 1994, *D. geminata* spread to several tributaries of the River Hvítá (Figure 2) and became a nuisance in many of them. Also in 1994, the species was reported in the River Elliðaár, in Southwest Iceland. During the following two years (1995 and 1996), *D. geminata* was reported from six other streams in South, West and Northwest Iceland (Figure 3). During this period there were no systematic surveys conducted on the algae in Icelandic rivers, except in the Borgarfjörður area (Jónsson et al. 1997). Within an individual stream system, *D. geminata* colonized the lower reaches first and spread upstream in subsequent years. No records are available of earlier presence of the species in Iceland (Jónsson et al. 1998).

A nationwide survey was initiated in 1997 to assess the distribution of the algae. The results showed that the diatom was mainly distributed in South, West and Northwest Iceland and that its distribution was not correlated to bedrock geology or conductivity (Jonsson et al. 2000). Even though the relative number of *D. geminata* varied between 0.1 and 4.2 % of the total number of diatom cells, this value corresponded to 16 - 85% of the total diatom cell volume (Jonsson et al. 2000), due to the large cell size of *D. geminata* compared to other diatom species.

A second national survey of *D. geminata* was carried out in 2006 (Jónsson et al in press). Samples were collected from sites in lowland streams, mainly in the Northwest and eastern part of Iceland. The results revealed that *D. geminata* now occurs in parts of the island, where it did not exist in the survey nine years earlier (Jónsson et al. 1998). *D. geminata* seems, however, to be less common and the occurrence less prominent in the rivers of Tertiary basalt bedrock formations (i.e. in the far West- and East of Iceland) than in streams of other bedrock types. It should be kept in mind that rivers in the highlands of central Iceland are poorly represented in these surveys.

Figure 2. The distribution of *Didymosphenia geminata* in the Borgarfjörður streams 1994 (red), 1995 (green) and 1996 (blue) (based on map from Jónsson et al. 1997).

In some streams of the Borgarfjörður area, where the intensity of *D. geminata* was high in the first years of its colonisation, the benthic coverage decreased and did not form any sign of nuisance blooms. In other sites, the coverage of the alga was similar in appearance to the high abundances that were first observed.

At the upper region of the River Grímsá, a tributary of the River Hvítá in Borgarfjörður, *D. geminata* was first reported in 1996 and has since been found there in a high density (Figure 2). No obvious sign of decrease in the densities of juvenile salmon has been observed in concordance to the presence of *D. geminata*, based on electrofishing data dating to 1991.

Figure 3. The locations of streams in Iceland where *D. geminata* occurred 1994 – 1996, marked with red, green and yellow dots. The red dot represents the River Elliðaár, near Reykjavík. The shaded box to the left is the Borgarfjörður area (based on data from Jónsson, et al. 1997 and 1998).

The effects of *D. geminata* on the stream flora and fauna in Iceland have not yet been thoroughly investigated. Although fish populations are influenced by other biotic and abiotic parameters, a negative impact of *D. geminata* on fish is not apparent in Icelandic streams. An ecological study on the possible effect by the diatom e.g. in areas of different abundance with focus on its effect on the stream benthic communities and fish stocks are essential.

SUMMARY

- After *D. geminata* was first recorded in Iceland, early in the nineties, it rapidly spread to neighbouring rivers and then to more distant rivers.

- The initial discovery of *D. geminata* occurred in streams of West and Southwest Iceland. The diatom has expanded its geographic distribution to lowland rivers in most regions but with differences in abundance between regions.

- In Borgarfjörður streams and in the River Elliðaár, the coverage of *D. geminata* was extensive with early colonization. The coverage seems to have decreased in recent years.

- No negative influence has been observed on fish stocks in Icelandic rivers.

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RELATIONSHIP BETWEEN NUISANCE BLOOMS OF *DIDYMOSPHENIA GEMINATA* AND MEASURES OF AQUATIC COMMUNITY COMPOSITION IN RAPID CREEK, SOUTH DAKOTA

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Nuisance blooms of *Didymosphenia geminata* are increasingly reported by the public and media, but little is known about its ecological impacts. Nuisance blooms have been observed in Rapid Creek, South Dakota throughout approximately 20 stream miles between two reservoirs, Pactola Reservoir and Canyon Lake, since June 2002. These recurring blooms persist for several months of the year and can cover a majority of the stream bottom (a maximum of approximately 80% coverage was observed at one monitoring site). Masses of stalk material have been reported by observers as being unsightly and are often mistaken for raw sewage. Homeowners along the affected reach of Rapid Creek also complain of stalk material obstructing pump intakes for their irrigation systems.

D. geminata blooms are suspected to have a negative effect on fish populations, especially fish that inhabit benthic habitats or consume benthic organisms (Spaulding and Elwell, 2007). Historically, Rapid Creek was considered one of the most productive trout fisheries in the western United States, averaging approximately 25 g/m^2 in Rapid City, South Dakota. More recently, the brown trout population has experienced significant declines in adult fish with an abundance of young-of-year and age-1 brown trout. This distinct "bottlenecking" of the brown trout population coincided with the appearance of *D. geminata* (Figure 1). However, it should also be noted that stream flows have decreased in recent years due to drought conditions, so the declining brown trout population could also be attributed, in part, to the reduced stream flow conditions in Rapid Creek.

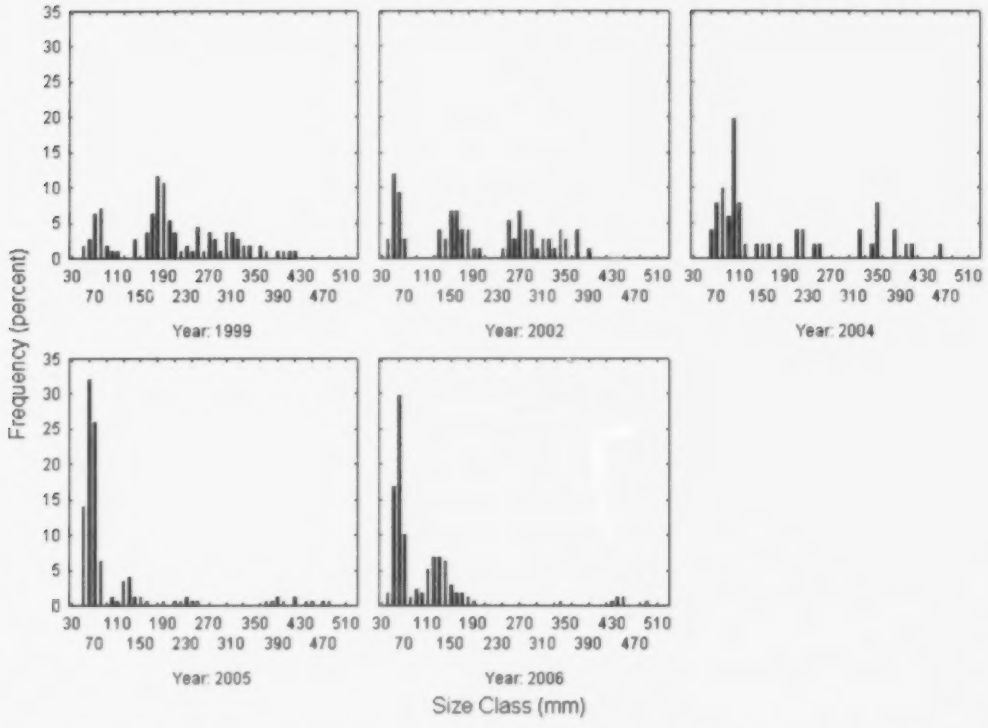

Figure 1. Fish size class distributions for fish surveys conducted for Rapid Creek below Pactola Reservoir in 1999, 2002, 2004, 2005 and 2006 (South Dakota Department of Game, Fish and Parks, unpublished data.

In response to the declining brown trout fishery, a nutrient enrichment project was initiated in May 2007 in Rapid Creek near the outlet of Pactola Reservoir. The goal of this project was to determine if primary and secondary productivity can be stimulated and, ultimately, the growth and survival of the abundant juvenile fish. A slow-release granular fertilizer was used to increase phosphorus concentrations and attain nutrient ratios (N:P) near the Redfield ratio of 16:1 (Redfield et al., 1963) by additions. Average total nitrogen and total phosphorus concentrations in that stream reach are 0.350 mg/L and 0.011 mg/L, respectively. Average TN:TP ratio in Rapid Creek is 31:1, which is considered a phosphorus-limited system (SD Department of Environment and Natural Resources, unpublished data).

Water quality and biological monitoring will be conducted at six sites through the duration of the enrichment project, which is scheduled to end in September 2009. Preliminary results suggest that primary productivity is enhanced by the nutrient additions, particularly at sites PCHC and PCHD (Figure 2).

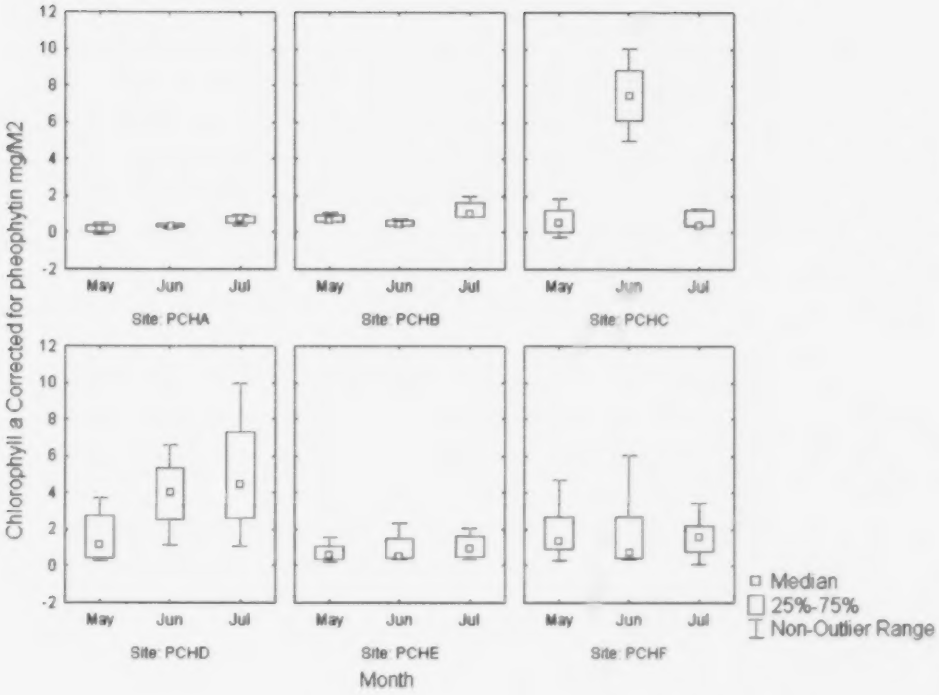

Figure 2. Chlorophyll *a* concentrations during the months of May, June and July 2007 (site names in alphabetical order going downstream; nutrient additions occurred between sites PCHA and PCHB) (South Dakota Department of Game, Fish and Parks, unpublished data).

Prior to the enrichment project, a separate stream assessment was conducted to examine the relationships between *D. geminata* blooms and other benthic (stream bottom-dwelling) organisms. Biological and water quality samples were collected semi-monthly from May through October of 2005 and 2006 at five monitoring sites.

Several benthic macroinvertebrate and algal metrics were correlated with visual estimates of *D. geminata* areal coverage of the benthos, suggesting that impacts to biological communities are directly related to the spatial extent of nuisance blooms. One of these macroinvertebrate metrics, the percent relative abundance of species within the orders Ephemeroptera, Plecoptera, and Trichoptera (percent EPT) shows a strong inverse relationship to *D. geminata* coverage (Figure 3). The lowest abundance of EPT (<10%) is found at the sites with the greatest areal coverage of *D. geminata*. As a group, EPT are generally large macroinvertebrates as compared to other macroinvertebrate organisms. The reduction, or loss, of EPT taxa may have consequences for the trout fishery, especially the larger fish, since the individuals prefer to feed on larger invertebrates.

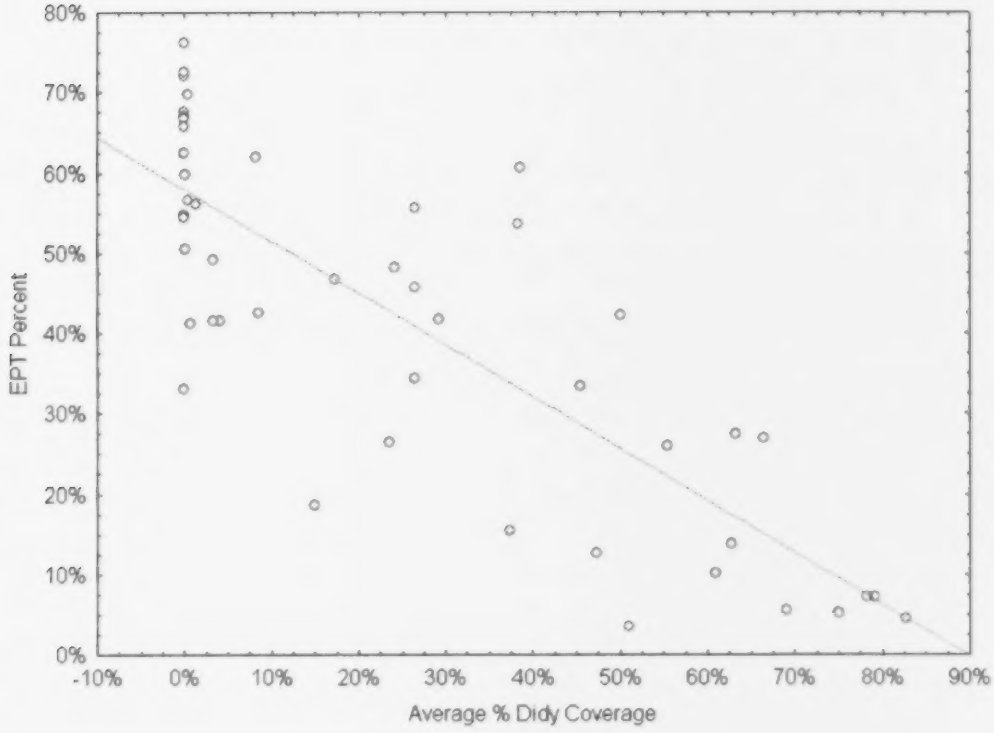

Figure 3. Average percent areal coverage of *D. geminata* compared to relative abundance of EPT taxa.

Overall, macroinvertebrate community structure at sites impacted by *D. geminata* blooms were less even and less diverse than at non-impacted sites. In addition, abundance and diversity of relatively large macroinvertebrates, such as Odonates, were inversely related to coverage of blooms. The orders Ephemeroptera and Trichoptera were significantly more abundant and taxonomically rich at non-impacted sites than at impacted sites. These macroinvertebrates appear to be replaced by more tolerant, and relatively smaller, midges and aquatic worms at impacted sites. Losses of larger invertebrate species at impacted sites may have contributed to the significant reduction of adult brown trout.

Results of this assessment indicate that nuisance blooms of *D. geminata* are associated with altered (degraded) macroinvertebrate communities. We expect that higher trophic level organisms, such as brown trout, are affected by loss of favorable food sources of large benthic macroinvertebrates in Rapid Creek.

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DID BLOOMS OF *DIDYMOSPHENIA GEMINATA* AFFECT RUNS OF ANADROMOUS SALMONIDS ON VANCOUVER ISLAND?

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The first major bloom of *Didymosphenia geminata* (didymo) documented in North America began in 1989 in the Heber River, a tributary of the Gold River on Muchalat Inlet, Vancouver Island, British Columbia (Sherbot and Bothwell 1993). During this episode, algal biomass in the Heber River greatly exceeded any previously recorded values for rivers on Vancouver Island. After 1989, didymo rapidly spread between rivers on south central Vancouver Island and by 1994, blooms had appeared in the mainstems of twelve rivers (Bothwell et al. 2006). Nearly all of the didymo-infested rivers were popular steelhead fishing sites in less populated areas of Vancouver Island.

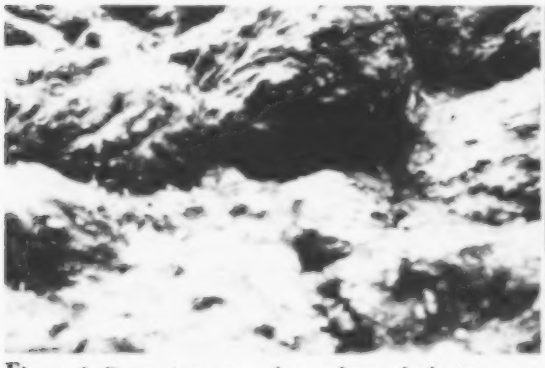

Figure 1. *D. geminata* covering rocks on the bottom of the Heber River - September 1989. Chlorophyll levels were ~1000 mg/m^2. (photo by J. Deniseger)

Figure 2. Typical river habitats for *D. geminata* on Vancouver Island were mainstem, rocky-bottomed river channels passing through forest corridors in remote areas often popular with anglers (photo by J. Deniseger)

The magnitude of the trophic level shift that occurred in these rivers was alarming to local authorities, concerned that the increased algal production might compromise habitat suitability for rearing juvenile salmonids by altering food webs and potentially harming deposited eggs by depressing dissolved oxygen concentrations within benthic gravels. During the early 1990's such concerns were frequently voiced in the local news media (Figure 3).

Mysterious growth of algae has biologists fearing for fry

By Richard Watts
Times-Colonist staff

The mysterious blooming of a golden brown algae in Vancouver Island streams and rivers has provincial biologists concerned about the survival of steelhead fry.

Lloyd Erickson, environmental section head for Vancouver Island, said it appears the recently discovered algae might affect the numbers of steelhead fry in a river, although no numbers have been worked out yet.

"There is a possible correlation between the number of steelhead fry and the algae . . . but we have yet to quantify it," said Erickson.

He also said insect survival — a food source for the young fish — might be affected by the appearance of the algae.

"We are concerned that there might be some problems with the food chain. It might inhibit insects in the vicinity," said Erickson.

He said the golden brown algae, called *Gomphonema geminata*, was first spotted on Vancouver Island about three summers ago.

Since then it has been found mostly in the mid-section of the island, on the Heber, Little Qualicum, Puntledge, Stamp and Somass Rivers.

The algae covers the rocks in mats where the coverage can range from spotty to nearly 100 per cent of the river bottom. These mats can grow up to three centimetres in thickness.

Erickson said nobody knows yet what has caused the sudden growth of the algae. It has not yet been linked to any pollution or unnaturally introduced substances.

"We are getting excessive algae growth in the absence of any excess nutrients," he said.

The algae is not poisonous, although one swimmer complained of itchy eyes after swimming in a spot where the algae was present.

Also, last year in Gold River water became foul tasting when the algae upstream died off in the fall, came off the rocks and entered the drinking-water system.

"But any algae that is dying and sloughing off the rocks can do that," said Erickson.

He said provincial scientists are now just trying to map out the algae's location and the extent of its infestation.

"We haven't really got a clear picture yet," said Erickson.

Figure 3. Article appearing in the Victoria, British Columbia Times-Colonist newspaper (July 17, 1991) outlines concern among Provincial biologists about potential impacts of didymo on salmon fry and juveniles. (Note: when *Didymosphenia geminata* blooms were first encountered on Vancouver Island it was occasionally referred to as *Gomphonema*, a genus the species had been previously included in)

For unknown reasons, the *D. geminata* blooms in many of these rivers waned between 1996 and 1999. By the early 2000's, blooms were no longer reported in many of the previously impacted rivers, although blooms still recur in some sites.

To determine whether *D. geminata* blooms had an impact on fisheries resources on Vancouver Island in the period 1989-1996, we examined long term data records on three species of Pacific salmon; coho salmon (*Oncorhynchus kisutch*), chum salmon (*O. keta*), and steelhead trout (*O. mykiss*). All three species rear in rivers that were impacted by didymo and all species of Pacific salmon are anadromous. These fish reproduce in freshwater, rear in the ocean and then return to their natal stream to spawn. Nearly all of the growth in these species occurs in the ocean and most of the variability in the abundance of adults results from changes in oceanic conditions (Geiger et al. 2002). The covariance of ocean survivals among adjacent populations is quite high over scales of 100 km. Consequently, it should be possible to detect effects of didymo in freshwater using by examining the abundance of adults through time series of adult abundance.

Extensive data sets of escapement (spawner abundance in natal stream) are available for chum and coho salmon of Vancouver Island streams, which allowed us to select time series for both didymo and non-didymo affected rivers. Together with regional indices of fisheries exploitation and known population age structure, we determined a productivity index, recruits per spawner ($R \cdot S^{-1}$) for each population. A regional composite time series of $R \cdot S^{-1}$ was generated for each species for rivers not impacted by didymo. We compared $R \cdot S^{-1}$ (adjusted for S) for the same species in didymo-impacted rivers with these regional composite values for pre-, during and post-didymo periods. We also compared the number of spawners (S) in rivers affected by *D. geminata* to the regional composite values for rivers not impacted by didymo.

In the five chum salmon rivers affected by *D. geminata*, the number of spawners was significantly higher during years of didymo infestation compared to didymo-free rivers. In four of those rivers there was no statistically significant ($P<0.1$) change in chum productivity ($R \cdot S^{-1}$) after adjusting for S. Escapements increased significantly ($P<0.001$) in two of the five coho salmon rivers affected by didymo but was unchanged in the other three rivers. Productivity increased significantly ($P<0.05$) in three of the five coho salmon rivers during the didymo blooms and was unchanged in the other two rivers. The analyses of chum and coho salmon escapement and productivity suggest that didymo infestation of rivers on Vancouver Island had either no detectable impact or, in some cases, a positive effect on salmon productivity.

There are few reliable estimates of steelhead abundance so analyzing trends for that species required a different approach. The Gold River and its major tributary, the Heber River, both support comparably sized steelhead runs. Both populations enter the ocean through a common estuary and are presumably exposed to the same oceanic conditions and ocean fisheries. Consequently, differences in their population trajectories or productivities are likely the result of differences in their respective freshwater environments. Accurate escapement estimates are available for both populations and during the period of record, the Heber River was affected by didymo while the Gold River was not. We compared the $R \cdot S^{-1}$ adjusted for spawner density of steelhead populations in the Gold and Heber Rivers in pre-, during, and post didymo periods. Those analyses indicated that steelhead productivity was the same in didymo and non-didymo rivers both before and during the period of *D. geminata* blooms.

For chum, coho and steelhead, our analyses indicate that *D. geminata* did not diminish the natural fish production capacity of rivers on Vancouver Island during a period of extensive blooms in the 1990's. Evidence suggesting possible stimulation of coho production associated with didymo was not consistent across all didymo affected rivers.

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MANAGEMENT APPROACHES TO DIDYMO: THE NEW ZEALAND EXPERIENCE

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In New Zealand, the legal basis for excluding, eradicating and effectively managing pests and unwanted organisms is provided under the Biosecurity Act 1993, administered by the Ministry of Agriculture and Forestry (MAF). Responses to risk organisms are led or coordinated by MAF Biosecurity New Zealand where the impacts pose nationally significant threats to New Zealand's people, environment or economy and/or where there is a need for rapid response to mitigate impacts. Responses aim to achieve the best overall outcome for New Zealand by minimizing the impacts of both the risk organism and the response itself. A portfolio approach is taken to response management, and responses are delivered in accordance with available resources and overall biosecurity priorities. Other government agencies, regional councils and pest management agencies can also use powers under the Biosecurity Act to manage risk organisms of importance to their constituents.

When the non-indigenous alga *Didymosphenia geminata* (didymo) was first discovered in New Zealand in October 2004, its invasive characteristics in the affected river system along with anecdotal evidence from overseas led to an assessment that the organism was likely to have considerable impacts on New Zealand's freshwater environment by affecting recreational, biodiversity, cultural and economic values. The focus of MAF Biosecurity New Zealand's response was determined by the practicalities of managing a freshwater incursion of an organism that is not always visible to users and by the general lack of information on impacts and response options globally.

An extensive MAF-sponsored science program has reduced uncertainties underlying response decision-making, as well as provided operational tools to manage the risks of the organism. The development of the 'Check Clean Dry' method for decontaminating gear used in affected waterways along with the strong social marketing campaign to encourage freshwater users to change their behaviour is likely to have slowed the spread of didymo around New Zealand. Didymo is currently found in 14 major river and lake systems within the South Island, but has not been detected in the North Island.

An economic impact assessment estimated the expected total present value impacts of didymo, in the absence of government intervention, to be $158 million over the eight years post discovery. It is estimated that even a two year delay to the arrival of didymo in the North Island would reduce the economic impacts by between $5 million and $62 million over the extended period of 10 years. There is currently no established method to eradicate or suppress didymo within

affected waterways. Work undertaken to date and the program for on-going management is therefore aimed at reducing the risk of didymo being introduced to the North Island as well as slowing its spread around the South Island.

MAF has developed a program with partners, including the Department of Conservation, local government, Fish and Game New Zealand, specific Maori entities and industry, that sets out future management arrangements and activities for didymo. A partnership approach to managing didymo, where everyone is taking responsibility and working collaboratively, should also reduce the spread of new and other existing aquatic pests.

29 June 2007

Didymosphenia geminata Sample Request

Background: The expanding global nature of *Didymosphenia geminata* (didymo) has prompted a genetic survey of the invasive alga's current distribution around the world. The purpose of this work is to elucidate a genetic marker that can be used to compare global populations in an effort to establish the likely origin of the New Zealand strain(s). In addition, the survey will provide vital clues about the spread and genetic variation of didymo throughout the world and possibly assist in the identification of biocontrol agents from native populations.

Professor Craig Cary, University of Waikato, is the lead investigator for the didymo genetic detection study being conducted in New Zealand, funded through Biosecurity New Zealand (http://www.biosecurity.govt.nz/pest-and-disease-response/pests-and-diseases-watchlist/didymosphenia-geminata/science-technical#detec). In an effort to conduct a global population genetic study of didymo, we are asking everyone, including attendees of the 30th Congress of the International Society of Theoretical and Applied Limnology (12 – 18 Aug 2007) and the International Workshop on *Didymosphenia* (18 - 19 Aug 2007) being held in Montreal, Canada to donate ethanol-fixed *D. geminata* samples to Prof. Cary. We intend to provide all those interested with the results of these analyses. We are especially interested in acquiring samples from the broadest distribution possible, including but not limited to samples from Canada, United States, Greenland, Iceland, Ireland, Scotland, England, Norway, Sweden, Finland, Estonia, Latvia, Lithuania, Russia, Belarus, Poland, Ukraine, Czech Republic, Slovakia, Austria, Switzerland, Hungary, Slovenia, Croatia, Serbia, Montenegro, Romania, Turkey, Kazakhstan, Afghanistan, India, Nepal, Bhutan, Myanmar, China, Mongolia, and North Korea.

Sampling Protocol: By placing *Didymosphenia* in ethanol, the organism is no longer viable and therefore will not pose a biosecurity threat during transport and importation. Please collect a clean (outer pigmented area of colonies) sample (1-2 gm wet weight) and immerse in 10 v/v of 70% ethanol for at least six hours. Excess ethanol can be decanted prior to transport, but leave at least the minimum volume required to fully immerse the sample. If ethanol is not available, any high grade clear alcoholic beverage (ie. vodka) will suffice. Room temperature storage is fine. Please use clean (sterile if possible), tightly sealed, screw-capped vials made from an alcohol-resistant plastic (polypropylene). Clearly label both the tubes and lids and provide accompanying documentation noting that the samples are *Didymosphenia geminata* stored in ethanol (or vodka). Note the sample location (name of river, GPS coordinates or approximate latitude-longitude, closest mapped town, country), site description, date collected, and contact details of person responsible for the collection. Tape the lids with parafilm or electrical tape. Wrap an absorbant paper towel around the tubes and transport in a plastic bag. If travelling by air, be prepared to stow the samples in your checked baggage. Whether you are accompanying the samples to Montreal or sending them directly to New Zealand, please follow the above protocol and include a copy of this letter.

We thank you for your contribution to this effort.

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